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

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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 neurons. 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 :

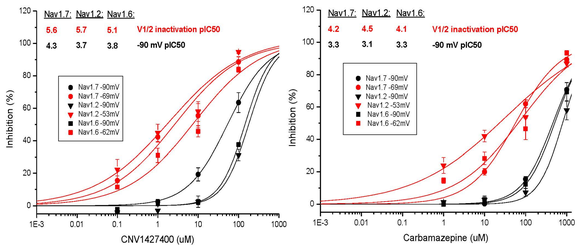
DRGs were obtained from neonatal rats (P7-10). Animals were euthanized in accordance with the 1986 Animals (Scientific Procedures) Act (Schedule 1). DRGs were dissected and dissociated by a prolonged collagenase treatment (0.1% in HBSS for 50 minutes at 37°C), followed by mechanical dissociation of the 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 neuroplegic 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.

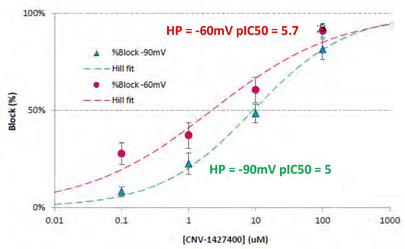
Results

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

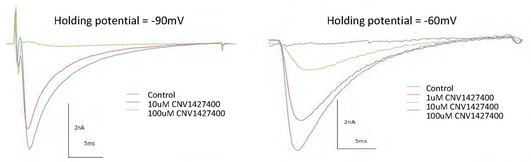


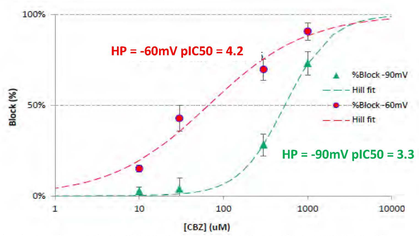
20ms pulses to 0mV delivered at 0.1Hz from a holding potential of -90mV (black curves) or V1/2 steady-state inactivation potential for each channel (red curves). External solution (in mM): NaCl 128, KCl 5, $MgCl_2$ 2, $CaCl_2$ 2, glucose 30 and HEPES 15 . Internal solution (in mM): CsCl 140, $MgCl_2$ 4, HEPES 10, EGTA 10, $CaCl_2$ 0.1, Creatine 2, Phosphocreatine diNa 2 and ATP-Mg 4. Recordings made at RT. N ≥ 3 cells per concentration.

CNV1427400 and Carbamazepine show a similar potency and mechanism of state-dependent block of Nav current in human native DRGs



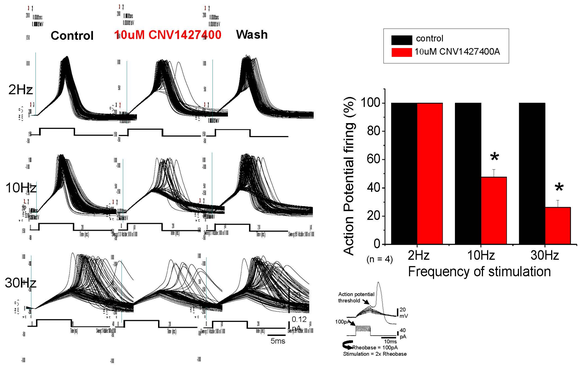
Protocol:
20ms pulses to 0mV, delivered at 0.1Hz from a holding potential of -90mV (green curves) or -60mV (red curves)





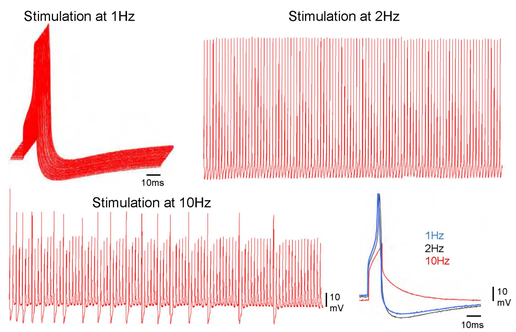
External solution (in mM): NaCl 150, BaCl 4 CaCl2 1.8, MgCl2 1, HEPES 10, Glucose 10 and CdCl2 0.05. Internal solution (in mM): NaCl 10, CsF 140, MgCl2 10, HEPES 10, EGTA 5. Recordings made at RT. Cells were obtained from 3 donors.

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

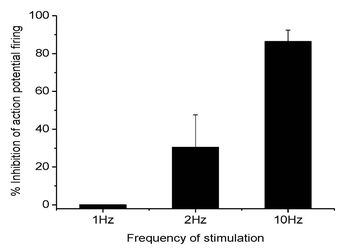


External solution (in mM): NaCl 124, KCl 3.4, MgCl2 1.2, CaCl2 2, Glucose 10 and HEPES 1.25, KH_2PO_4 1.2, $NaHCO_3$ 26. Internal solution (in mM): K-Gluconate 135, KCl 5, MgCl2 2, HEPES 5, EGTA 5, CaCl2 0.5, ATP-Mg 5, Na-GTP 0.5. Recordings made at 32 degrees C. N ≥ 3 cells per frequency.

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



Stimulation intensity = 2 x rheobase, 100 stimuli at 1, 2, and 10Hz



N = 6 cells / frequency from 3 different donors

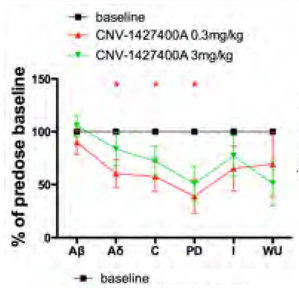
External solution (in mM): NaCl 145, KCl 3, CaCl2 2, MgCl2 2, HEPES 10, Glucose 10. Internal solution (in mM): K-gluconate 130, NaCl 5, KCl 5, HEPES 10, Sucrose 20, EGTA 0.3, MgATP 3. Recordings made at 32 degrees C, in oxygenated ACSF.

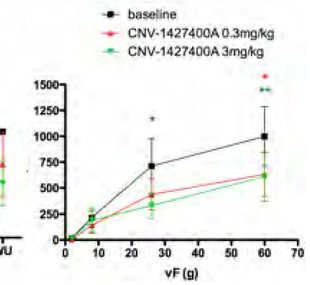
CNV1427400 – broad efficacy across pain models and wide therapeutic index

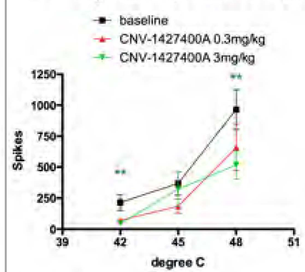
In vivo Model / behavioural test	Data	Exposure (unbound)
Acute inflammatory pain (intaplantar FCA)	MED 1mg/kg p.o. (*) (ED_{50} 0.6mg/kg p.o.)	13ng/ml
Chronic inflammatory pain (intra-articular FCA)	MED 1mg/kg p.o. (*), 10mg/kg p.o. (*), equivalent to Celecoxib 30mg/kg p.o.	--
Neuropathic pain (Chronic Constriction Injury)	MED 0.5mg/kg p.o. (*) equivalent to Gabapentin 30mg/kg p.o.	9ng/ml
Laboras (locomotor activity, rearing and grooming)	NOAEL 40mg/kg p.o.	485ng/ml

MED: minimum effective dose, NOAEL: no adverse event level, (*) statistical significance

At doses showing efficacy in pain models, CNV1427400 inhibits WDR neuron firing in SNL rats







- Recordings of WDR neurons from 7 animals which received SNL (spinal nerve ligation, Kim and Chung, 1992) surgery 14-18 days before use. All were confirmed as developing hindpaw hypersensitivity in behavioural testing at day 14 (Urch et al., 2003)
- Compound administered s.c. at 0.3 or 3mg/kg, the mean plasma Cmax exposures were 37 and 417ng/ml respectively
- The effect of the compound was tested at 10, 30 and 50 minutes post dosing

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.

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