Biophysical characterization of duloxetine activity on voltage-gated sodium channels involved in pain transmission

Xention

Jean-François Rolland, David Madge, John Ford, Marc Rogers
Xention Ltd, Iconix Park, London Road, Pampisford, Cambridge, CB22 3EG, United Kingdom

Xention

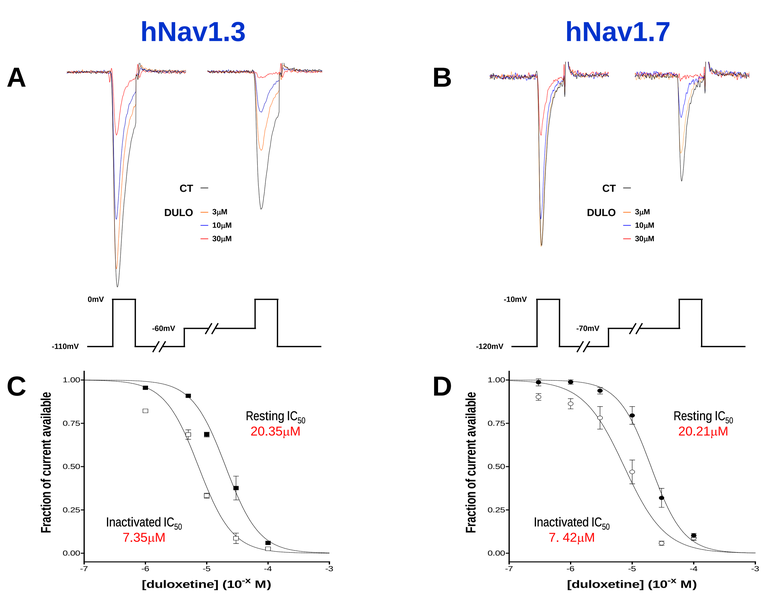
Introduction

In the U.S. 17 million adults have physician-diagnosed diabetes and 1.6 million new cases are diagnosed every year (www.diabetes.org); in England the prevalence is over 2 million (www.nhs.uk/Diabetes). From 16 to 26% of diabetes patients suffer from chronic neuropathic pain (Daousi et al., 2004) with symptoms including numbness, tingling or prickly sensations, and intense burning pain.

Duloxetine is an inhibitor of the serotonin-norepinephrine reuptake system widely used for treating major depression, and in 2004 became the first agent to receive FDA approval for managing pain associated with diabetic peripheral neuropathy. Its efficacy has been postulated to result from increased monoamine signalling in descending spinal and supraspinal nociceptive circuits, but the exact mechanism of analgesia is still debated. Such anti-nociceptive action is also observed with other antidepressants (amitriptyline, desipramine), and it is notable that many of these also exert use-dependent inhibition of voltage-gated sodium channels (Wang et al., 2004; Lenkey et al., 2006), suggesting that this mechanism may contribute to their anti-nociceptive action. However, the possible effect of duloxetine on sodium channels has never been investigated, and in fact it has been claimed not to affect any other ion channels or receptors known to be involved in pain signalling.

Thus, we investigated its potential effect on two neuronal isoforms of voltage-gated sodium channels (Nav1.3 and Nav1.7) with pivotal roles in the generation and propagation of pain signals (Rogers et al., 2006). State and use-dependent inhibition was assessed to reflect the possible effects of duloxetine on the activity of sodium channels during high frequency discharges and prolonged membrane depolarization characteristic of nociceptive transmission. In addition to gain a better understanding of the therapeutic action of duloxetine, this study may also provide insights into the structural requirements for the design of new selective sodium channel blockers.

State-dependent Block



Duloxetine inhibits both Nav1.3 and Nav1.7 channels.
Duloxetine exhibits a higher affinity for the inactivated state than for resting state (ratio resting IC_{50} to inactivated IC_{50}: 2.77 and 2.72, for NaV1.3 and NaV1.7, respectively)
A - **B**: Representative hNav1.3 (A) and hNav1.7 (B) current traces are superimposed (before -black trace- and after application of duloxetine, 3, 10 and 30 μM -orange, blue and red traces, respectively).
C - **D**: Concentration-response curves for the inhibitory effects of duloxetine on resting (closed symbol) and inactivated (open symbols) hNav1.3 (C) and hNav1.7 (D). Each point represents the mean SEM of 3 to 12 experiments.

Methods & Materials

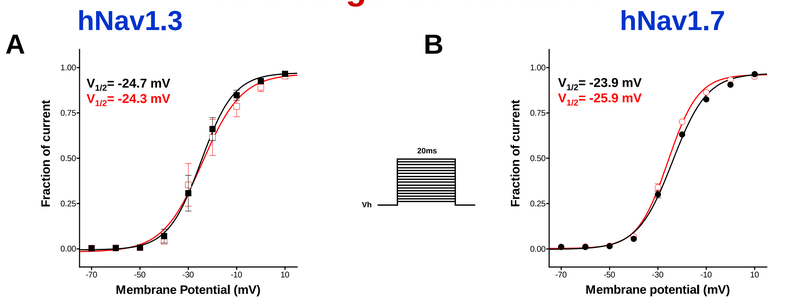
Tissue culture
CHO and HEK293 cells stably expressing hNav1.3 and Nav1.7, respectively, were maintained in media containing 10% FCS and appropriate selection antibiotic. Cells were either grown in suspension or T-175 flasks and routinely passaged. Cells for patch-clamp experiments were plated onto petri dishes prior to use.

Conventional patch-clamp electrophysiology
Standard gigaseal whole-cell patch-clamp techniques were performed at room temperature (22°c) using glass pipettes (2-3 MΩ). Pipettes were filled with an intracellular solution of the following composition (mM): 15 NaCl, 120 CsF, 10 HEPES, 10 EGTA (pH: 7.25). The composition of the external solution was (mM): 140 NaCl, 5 KCl, 1 CaCl2, 1.2 MgCl2, 5 HEPES, 11.1 Glucose (pH: 7.4). HEKA EPC amplifiers and Pulse software were used for data acquisition. Series resistance was compensated by >30%. Voltage protocols are reported in the appropriate figures.

Compounds
Duloxetine was dissolved and serially diluted in DMSO. Final DMSO concentration in experiment solution was either 0.1 or 0.3 %.

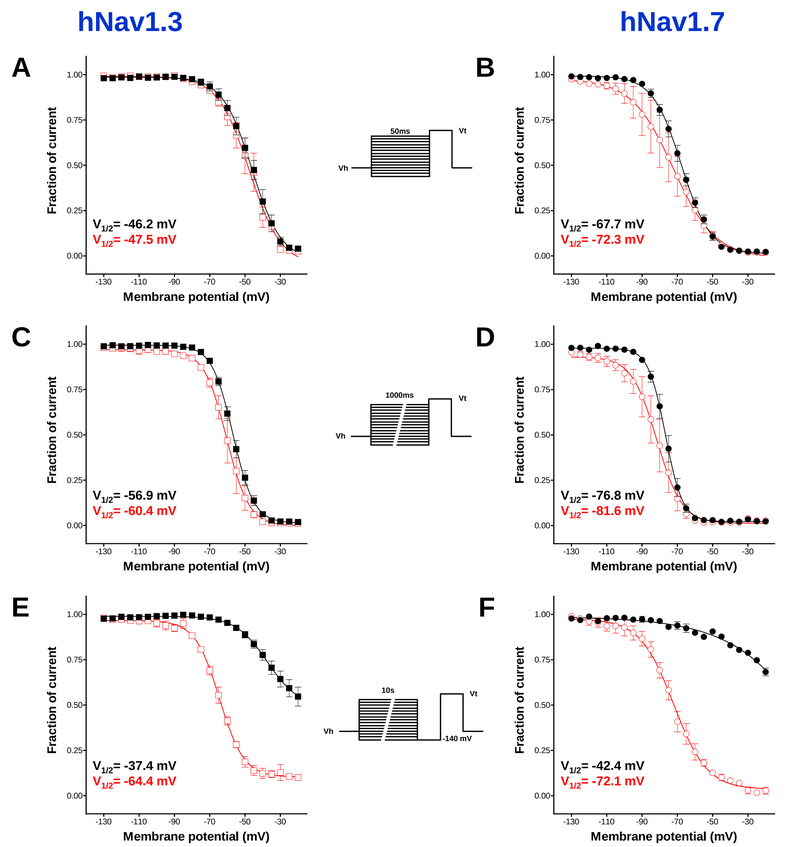
Analysis
Data are expressed as means (± SEM).

No Change in Activation



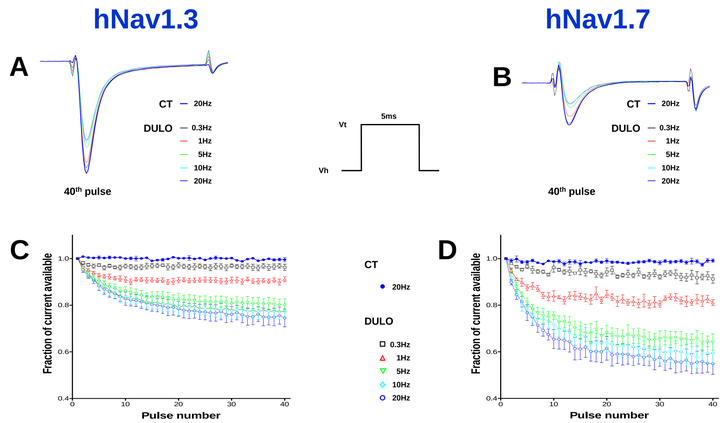
Duloxetine does not alter the voltage-dependence of activation of either hNav1.3 or hNav1.7 currents.
A - **B**: Activation curves of hNav1.3 (A) and hNav1.7 (B) in the absence (black closed symbols) and presence of 10 μM duloxetine (red open symbols). Vh=-110 and -120 mV, for hNav1.3 and hNav1.7, respectively. Each point represents the mean SEM of 7 to 12 experiments.

Stabilization of Inactivated State



Duloxetine modifies the voltage-dependence of slow inactivation of both hNav1.3 and hNav1.7 currents.
The effects of duloxetine (10μM) on the voltage-dependence of steady-state fast, intermediate and slow inactivation (upper, middle, and bottom panels, respectively) for hNav1.3 and hNav1.7 (right and left panels, respectively) were evaluated using the indicated voltage protocols (middle panels, Vh=-110 and -120 mV and Vt=0 and -10mV, for hNav1.3 and hNav1.7, respectively). Each point represents the mean SEM of 4 experiments.

Use-dependent Block



Duloxetine inhibits both hNav1.3 and hNav1.7 channels in a use-dependent manner.
A - **B**: Representative overlaid hNav1.3 (A) and hNav1.7 (B) current traces of the 40th pulse in control conditions (thick blue trace, 20Hz) and in the presence of duloxetine (10μM; 0.3, 1, 5, 10 and 20Hz; black, red, green, cyan and blue thin traces, respectively). Vh=-110 and -120 mV and Vt=0 and -10mV, for hNav1.3 and hNav1.7, respectively.
C - **D**: Plot summarizing the frequency-dependent decrement in peak hNav1.3 (C) and hNav1.7 (D) current amplitude in control conditions (20 Hz, blue closed symbol) and in the presence of duloxetine (10μM; 0.3, 1, 5, 10 and 20Hz, black, red, green, cyan and blue open symbols, respectively). Each point represents the mean SEM of 4 experiments.

Summary

- The present study provides the first demonstration that duloxetine inhibits in a concentration-dependent manner both hNav1.3 and hNav1.7, sodium channels involved in pain transmission.
- Duloxetine exerts a state-dependent block of both channels, selectively stabilizing the slow-inactivated state. No significant effect is observed on fast/intermediate inactivation, nor on the voltage-dependence of activation.
- Duloxetine also exhibits use-dependent block, which is more pronounced for hNav1.7 than hNav1.3.

Conclusion

As a significant inhibition of both hNav1.3 and hNav1.7 currents occurs above the predicted plasma concentration of duloxetine during clinical dosing, it would appear that block of sodium channels is unlikely to contribute to its antinociceptive action. However, several arguments can be made for a role of sodium channel modulation by duloxetine:

- The therapeutic effect of antidepressants appears at much higher plasma and brain concentrations than the nanomolar IC_{50} against the monoamine transporters (Torres *et al.*, 2003).
- Sub-micromolar concentrations of duloxetine appeared to produce persistent inhibition of the inactivated state of Nav1.7 currents.
- A small reduction in the fraction of available sodium channels can significantly affect nociceptive neuronal firing (Ritter *et al.*, 2006).
- The effect of duloxetine could be greater in native tissues, due to the effect of auxiliary subunits as well as the facilitatory action of other pain mediators on channel biophysics (e.g. inflammatory mediators, channel phosphorylation).

The results also add information about structural features and mechanisms of action that may aid in the development of new sodium channel blockers for treating neuropathic pain.

References

Daousi *et al.*, Diabetic Medicine, 2004.
Lenkey *et al.*, Molecular Pharmacology, 2006
Ritter *et al.*, The Journal of Pain, 2006.
Rogers *et al.*, Seminars in Cell & Developmental Biology, 2006
Torres *et al.*, Nature Reviews Neuroscience, 2003
Wang *et al.*, Pain, 2004.
www.diabetes.org
www.nhs.uk/Diabetes