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

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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 signaling in descending spinal and supraspinal nociceptive circuits, but the exact mechanism of analgesia is still unknown. 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 (Daousi 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 gaining 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 modifies the voltage-dependence of slow inactivation of both Nav1.3 and Nav1.7 currents.

Stabilization of Inactivated State

Using a modified voltage clamp protocol (Ritter et al., 2006), we could show that the steady-state fraction of Nav1.7 currents stabilizes at 0.3 Hz during high density nerve stimulation (Ritter et al., 2006). A clear time-dependent modification of this steady state inactivation is observed with both Nav1.3 and Nav1.7 currents, which is reflected by a decrease in the membrane potential associated with the inactivation process (0.3 Hz, 1 Hz, 5 Hz, 10 Hz, 20 Hz, black, red, orange, cyan and blue thin traces, respectively). Vth=-110 and -120 mV and Vt=0 and -10 mV, for Nav1.3 and Nav1.7, respectively. Each point represents the mean SEM of 4 experiments.

Conclusion
As a significant inhibition of both Nav1.3 and Nav1.7 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 the 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 minimal IC50 values reported for key sodium channels (Blaustein et al., 2004).
- A small reduction in the fraction of available sodium channels can significantly affect nociceptive neuronal firing and pain threshold.

Summary
- The present study provides the first demonstration that duloxetine inhibits in a concentration-dependent manner both Nav1.3 and Nav1.7, 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/inactivation, nor on the voltage-dependence of activation.
- Duloxetine also exhibits use-dependent block, which is more pronounced for Nav1.7 than Nav1.3.

Methods & Materials
- 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: whole-cell currents were recorded at room temperature using a headstage 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 MgCl2, 2 CaCl2, 10 HEPES, 10 glucose (pH: 7.4).
- Duloxetine was bath applied at final concentrations of 0.01, 0.1, 1, 5, 10, 50, 100, 500, 1000, 3000, 5000, 10,000, and 30,000 µM; 0.3, 1, 5, 10 and 20 Hz; black, red, orange, cyan and blue thin traces, respectively. Vth=-110 and -120 mV and Vt=0 and -10 mV, for Nav1.3 and Nav1.7, respectively.

References
- Daousi et al., Diabetic Medicine, 2004.