Addendum

Block of Na\textsubscript{v} 1.8 by Small Molecules

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ABSTRACT

Sodium channels are key proteins in regulating neuronal excitability and accumulating data suggest that specific subtypes of voltage-dependent sodium channels are important in signaling various types of pain. Consistent with this theme, Jarvis et al.\textsuperscript{7} recently reported the identification of a subtype-selective Na\textsubscript{v} 1.8 blocker that was active in several pre-clinical models of pain. During the course of these studies compounds were also identified that showed large differences in potency when tested on Na\textsubscript{v} 1.8 channels from different species. This addendum illustrates one of these compounds along with the potency correlation between recombinant and native tetrodotoxin-resistant sodium channels for additional examples. These data show that significant differences can be observed for sodium channel blockers across species and highlight the importance of considering this possibility when searching for new compounds and research tools to probe sodium channel function.

Sodium channel blockers are valuable therapeutics and remain one of the more effective classes of drugs for the treatment of pain.\textsuperscript{1} The proven value of small molecule therapeutics in this area coupled with the identification of nine genes encoding human sodium channel subtypes begs the question of whether or not small molecules can be identified which selectively block one sodium channel subtype over another. A follow-on question is whether such molecules are effective in ameliorating pain. Recently, strong genetic validation for the important role of one sodium channel in pain sensation has only increased the level of interest in this area.\textsuperscript{2-6}

We recently published the first report of a small molecule, A-803467, which selectively inhibits one sodium channel subtype\textsuperscript{7} and is also efficacious in a variety of pre-clinical pain models. As such this compound represents a potentially useful research tool. A-803467 blocks hNa\textsubscript{v} 1.8 with an IC\textsubscript{50} value of 8 nM under conditions where channels are at half-maximal inactivation. Comparable protocols run against other sodium channel subtypes demonstrated a selectivity ratio of 30–1000-fold for A-803467. One of the more interesting biophysical properties of this compound is that it blocks hNa\textsubscript{v} 1.8 channels at negative resting membrane potentials (e.g., -100 mV) and shows little if any use-dependent block during a 10 Hz pulse train. Typically, most small molecule sodium channel blockers show a much lower affinity for the resting state than the inactivated state of the channel. Our results suggest a significant interaction with the resting state of hNa\textsubscript{v} 1.8 channels for A-803467.

One observation noted in our paper was a potency shift when comparing native rat Na\textsubscript{v} 1.8 to human recombinant Na\textsubscript{v} 1.8. The compound demonstrated a -6-fold shift in potency between human and rat recombinant channels and a -3-fold shift compared to native currents from dissociated DRG neurons. During the course of our program we investigated this relationship for a variety of chemotypes and found varying degrees of potency ratios between rat and human Na\textsubscript{v} 1.8. Figure 1 illustrates concentration-response relationships for Na\textsubscript{v} 1.8 block of human, rat and native TTX-resistant currents. In this example, hNa\textsubscript{v} 1.8 block was essentially the same from either negative (-100 mV) or more depolarized membrane potentials (-40 mV), which are at the V1/2 for steady-state inactivation. However, when rat Na\textsubscript{v} 1.8 currents were studied, the same compound was 40-fold less potent against the rat channel from depolarized membrane potentials. Moreover, this shift was even greater from negative holding potentials where a -200-fold difference was observed. These differences could not be accounted for by the biophysical properties of

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Addendum to:
A-803467, a Potent and Selective Nav1.8 Sodium Channel Blocker, Attenuates Neuropathic and Inflammatory Pain in the Rat

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the channels since the gating parameters were similar. Effects on native TTX-r currents were also similar to those observed for rat Na\textsubscript{v1.8}.

Correlation plots between the various IC50 values measured across multiple compounds are shown in Figure 2 where results from recombinant currents are plotted in panel A and rat Na\textsubscript{v1.8} vs. TTX-r IC50 values in panel B. While many compounds fall close to the unity line when comparing rat and human Na\textsubscript{v1.8} currents, there clearly are compounds in this particular set which show a preferential interaction with the human channel. As one might expect, the correlation between rat Na\textsubscript{v1.8} and native TTX-r currents is reasonably good with the recombinant system being a good reflection of the native system.

Overall, one can draw a few interesting conclusions from these results. Since we observed differences in potencies for the same compounds tested against recombinant channels from different species, our data suggest actual differences in the binding sites for such compounds on the α-subunit. The rat α-subunit appears to be a good surrogate for the native rat channel in DRG neurons, however, and small differences observed on a compound-to-compound basis may simply reflect experimental variation. These results are particularly important in a drug discovery setting and emphasize the importance of determining pharmacology on both the human channel as well as the species of interest for pre-clinical in vivo models. Without these results in hand, data interpretation can be difficult or misleading with respect to development of structure activity relationships.

Given the degree of activity in the area of sodium channel research and pain drug discovery, one may anticipate that our results will be followed by others describing more research tools as well as next generation sodium channel blockers. In addition, one can only hope that this research will lead to a new generation of pain therapeutics.

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Figure 1. Concentration-response curves for block of Na\textsubscript{v1.8} and TTX-R currents by a test compound. Currents were measured for human Nav1.8 (squares), rat Na\textsubscript{v1.8} (circles) and TTX-R currents from DRG neurons (triangles). Currents were measured at test depolarizations to 0 mV following prepulse potentials to either -100 mV (open symbols) or -40 mV (closed symbols). Data are plotted as mean ± sem. Smooth curves were fits of a logistic equation to the data with minimum and maximum fixed at 0% and 100%, respectively.

Figure 2. Correlation plots for block of human vs. rat Na\textsubscript{v1.8} currents and rat vs. TTX-R currents. IC50 values were determined from experiments as outlined in Figure 1 using data from a -40 mV prepulse potential. Each symbol represents an individual compound. The same compound set is plotted in (A and B).