MEL55A is a novel blocker of hyperpolarization-activated (HCN) channels with isoform selectivity and therapeutic potential in the pain axis

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HCN channels have a primary role in the regulation of intrinsic excitability and rythmogenesis of cardiac sinoatrial node cells, central and peripheral neurons. Ivabradine (Procoralan or Corlentor) is the only HCN channel blocker on the market, approved as bradycardic agent in the treatment of stable angina and cardiac failure.
Emerging evidence document the involvement of HCN channels in extra-cardiac pathologies, including neuropathic pain, thus greatly enhancing the therapeutic potential of HCN blockers. Currently, the different molecules available for clinical use do not discriminate the different isoforms (HCN1/2/3/4), which form tissue-specific channel types, thus severely limiting the possibility to target selectively HCN channels in a distinct tissue and prevent the occurrence of adverse reactions.
We have recently identified new phenylalkylamine derivatives able to block HCN channels with potency comparable to that of reference compounds cilobradine and ivabradine, and showing isoform selectivity. Among them, preliminary experiments indicated that MEL55A (1) is more selective toward HCN2 isoform, leading to consider it as potential candidate as HCN current blocker in dorsal root ganglia (DRG) neurons, expressing mainly HCN2, which play an essential role in transmitting signals in the spinal cord (2).
The aim of this study was to characterize the isoform selectivity of MEL55A in recombinant HCN channel system and its electrophysiological properties in native HCN current expressed in DGR neurons.
Isoform selectivity was assessed by single-cell patch-clamp recordings in HEK293 cells re-expressing mHCN1, mHCN2 and hHCN4 channels. Effect on native current was studied in DRG neurons cultured for at least 24h from dissociation from mouse spinal cord. In the same cells action potential recordings were performed in the current clamp mode. Immunofluorescence microscopy was used to characterize the expression pattern and the localization of HCN channels in DRG neurons.
Electrophysiological recordings of recombinant HCN channel showed that MEL55A (10μmol/L) is able to cause a preferential block of HCN2 isoform, which at -80mV was reduced by 71% (0.17±0.07 vs 0.05±0.01 pS/pF, n=4-3). At the same concentration HCN1 was blocked by 66% (0.72±0.05 vs 0.24±0.07 pS/pF, n=4) and HCN4 by 33% (0.4±0.07 vs 0.27 ±0.07 pS/pF, n=4). In the same conditions, ivabradine (10μmol/L) confirmed the lack of isoform-selectivity, being HCN1 blocked by 51% (0.82±0.06 vs 0.4±0.08 pS/pF, n=5), HCN2 by 19% (0.21±0.04 vs 0.17 ±0.1 pS/pF, n=6); and HCN4 by 51% (0.42±0.07 vs 0.20 ±0.06 pS/pF, n=5). In DRG neurons MEL55A (10μmol/L) reduced current by 71% (0.26±0.06 vs. 0.08±0.05 pS/pF, n=7-4). Analysis of activation curve revealed that degree of current blockade was more pronounced at less negative potential (-80mV), a property that confirms the preferential block of HCN2, which contributes more to the current in DRG neurons. In the same cells, immunofluorescence staining revealed a strong expression of HCN2 at the membrane level that is associated with a less pronounced expression of HCN1. Finally, preliminary experiments in DRG neurons showed that MEL55A is able to increase the threshold of excitability, delay the latency and prolong the duration of action potential. Further investigations are necessary to establish the functional consequences of these effects on overall DRG neurons excitability.
Taken together these data encourage the use of MEL55A in animal model of neuropatic pain, where pain axis is regulated by DRG function and/or dysfunction; they also could pave the way for novel drug design of potential benefit in pain. The general conclusion is that the selectivity of HCN blockers is an affordable approach to target preferentially HCN function in a distinct tissue.

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