MEXILETINE AND TOCAINIDE ANALOGUES AS LEAD BLOCKERS OF NAV1.4CHANNEL: STRUCTURE-ACTIVITY RELATIONSHIP STUDIES AND POTENTIAL IMPLICATION FOR THE ANTIMYOTONIC ACTIVITY

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Introduction: The skeletal muscle sodium channel (Nav1.4) is the molecular target of current first-line therapies against myotonic-syndromes. Class Ib-antiarrhythmic lidocaine(LA)-like drugs, such as mexiletine, are used antimyotonic compounds because they block Nav1.4in a state- and use-dependent-manner. This blocking mechanism relies on the high-affinity drug binding to the channel in its open or inactivated state, and to a slow recovery from inactivation of the drug bound channel. These actions dampen abnormal membrane excitation and prevent early reexcitation during membrane repolarization. However, LA-like compounds can cause undesirable effects further reducing the few therapeutic options. It is of importance to deepen the understanding of the molecular requisites of drugs acting on Nav1.4, in terms of both absolute potency and state-dependent block to obtain more selective and safer antimyotonic drugs. For many years, we focused at clarifying the molecular determinants of LA-like drugs, in newly synthesized analogues of both tocainide (Toc) and mexiletine (Mex), providing useful information for docking-energy studies. We focused on new derivatives that have the following modifications at key pharmacophores: I) increased steric hindrance on the stereogenic-center; II) increased distance between the chiral carbon atom and the amino-terminal-group;. III) introduction of a tetramethyl-pyrroline moiety on the amino-group.

Methods: The compounds were tested on sodium currents of single frog skeletal muscle fibers by means of voltage-clamp-recordings. Steps to -20mV from the holding potential of 100mV at different stimulation frequencies(0.3up to 10Hz), were applied in order to evaluate tonic (TB) and use-dependent blocks (UDB) by drugs.

Results: We found that the presence of a lipophilic moiety (phenyl, benzyl group) on either the chiral center or the amino-group nearby enhances potency up to 30-fold. We investigated the effects of combination of the alkyl-chain elongation with the presence of an aromatic-group on the chiral center (Me12) or on amino-terminal-group (To40). S-Me12maintained the expected high potency for TB, the IC50 value being 10.5±0.5μM; however, it showed a greater UDB than parent compounds with a ratio IC50TB/IC50 UDB at 10Hz of 5.5. The presence of a benzyl-group on the nitrogen atom, as in To40, increased the potency for TB and mostly for UDB, compared to benzyl-Toc. The IC50 of To40 for UDB at 10Hz was 0.3±0.02μM, up to 30-fold lower than that of benzyl-Toc (De Bellis et al., Neuropharmacology 2017). We also investigated further structural changes by introducing a tetramethyl-pyrroline-ring on the amino-terminal-group of Mex (VM11) and of its potent use-dependent isopropyl-derivative (CI16). The pyrroline-group is expected to change the molecule physicochemical properties (logP and pKa), improving both the hydrophobic interaction with the binding-site and the use-dependent behavior, while possibly conferring new pharmacological activities, such as an antioxidant-action. Accordingly, VM11and CI16were 3and 6-fold more potent than Mex in producing a TB (IC50=23.4±0.9μM and 12.6±0.2μM, respectively). Both had increased use-dependent behavior, with CI16showing a 40-fold increase of potency versus Mex during high-frequency stimulation (IC50=0.6±0.1μM), resulting the strongest use-dependent Mex-like compound so far. VM11and CI16showed a remarkable cytoprotection in C2C12-myoblasts against H_2O_2-dependent-damage, at concentrations close to the IC50 for blocking Nav1.4. Then, the pyrroline-compounds have increased therapeutic profile as Nav1.4blockers and an interesting cytoprotective activity (De Bellis M. et al., Frontiers in Pharmacol. 2018).

Discussions and conclusions: The results reinforce our long-term objective to develop a reliable pharmacophore model for these compounds which may help to improve potency and selectivity toward Nav1.4for future therapeutic applications in rare diseases.