DEVELOPMENT OF NEW BENZOTHIAZOLAMINES TO ENHANCE USE-DEPENDENT INHIBITION OF VOLTAGE-GATED SODIUM CHANNELS.

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We have previously shown that human skeletal muscle voltage-gated sodium channels (hNav1.4 isoform) are potently inhibited by riluzole and lubeluzole, two benzothiazolamines (Desaphy et al., 2013). Both drugs also showed potent antimyotonic activity in an animal model of myotonia congenita, a skeletal muscle disease characterized by muscle fiber over-excitability and muscle stiffness (Desaphy et al., 2014). The sodium channel blocker, mexiletine, is currently the first-line drug for myotonic syndromes, but a number of patients remain unsatisfied with this drug. Riluzole is currently used in the treatment of amyotrophic lateral sclerosis, while lubeluzole has been tested in clinical trials for neuroprotective effects in stroke.

In the current study, we performed rationally designed chemical maneuvers on riluzole and lubeluzole in order to further exalt drug potency and use-dependence properties of sodium channel block. The new compounds were tested on sodium currents (INa) recorded using the patch-clamp method in HEK293 cells permanently transfected with hNav1.4.

We first focused on the trifluoromethoxy group (-OCF3) in riluzole. The deletion of this group or its substitution by a methoxy (-OCH3), an hydroxyl (-OH), or a chlorine (-Cl) greatly impaired INa inhibition. Instead, substitution by trifluoromethyl (-CF3) had no consequence for INa inhibition, and by isopropyl (-CH(CH3)2) only slightly reduced INa block. This first set of experiment suggests that the presence of an electron-donating group is deleterious for efficient INa inhibition. In contrast presence of a bulky and/or lipophilic group, such as isopropyl or trifluoromethyl, at the same position allows to maintain significant inhibition. Yet, similarly to riluzole, all the compounds tested show little use-dependence. We thus synthesized riluzole analogues with introduction of a more basic, protonable amine group. One of these compounds gained use-dependent behavior, showing INa inhibition similar to riluzole at 0.1 Hz frequency stimulation, but a two-fold increased inhibition at 10 Hz.

Regarding lubeluzole, one concern is its ability to prolong the QT interval of the ECG, which led to development arrest in previous clinical trials. We thus decided to synthesize a derivative, namely LUB1, with a reduced lipophilia by introducing hydroxyl groups in the two outermost aromatic cycles. Indeed, it is widely acknowledged that an increased lipophilia is generally associated with an increased inhibition of cardiac hERG channels, the molecular counterpart of QT interval duration. We previously demonstrated that the hydroxyl substitution in meta position of mexiletine aryl ring has no detrimental effect on INa blockade (Desaphy et al., 2012), while reducing the potency on hERG channels (Gualdani et al, 2016). INa inhibition by LUB1 was slightly less compared to lubeluzole. LUB1 was however more use-dependent than lubeluzole, because reduction of INa inhibition was more evident at 0.1 Hz stimulation frequency compared to 10 Hz stimulation. In conclusion, by using specific chemical maneuvers, we obtained derivatives of

riluzole and lubeluzole with enhanced use-dependent behavior. These compounds will serve as leads to design compounds with major selectivity toward over-excited tissues, which may prove useful in myotonic muscles as an alternative to mexiletine (project #19027 granted by Association Française contre les Myopathies).

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