Effects of mexiletine and its pyrroline derivative on $Na_V1.4$ and $Na_V1.5$ by automated patch-clamp: towards safer anti-myotonic drugs

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1. Introduction

Myotonia is an inherited rare skeletal muscle disorder which causes sarcolemma hyperexcitability due to alterations of different ion channels (e.g., CLC-1, $Na_V1.4$)[1]. Mexiletine is a class I antiarrhythmic drug approved in non-dystrophic myotonias for its ability to exert a use-dependent block of skeletal muscle sodium channel ($Na_V1.4$)[2,3]. Mexiletine showed to reduce muscle stiffness, weakness and pain in patients of two randomized controlled clinical trials[4,5] and to be safe in two long-term clinical studies[6,7]. Nevertheless, due to different side effects (e.g. gastrointestinal and cardiac problems, headache, dizziness, etc.) or lack of response, several patients are not eligible for mexiletine treatment[4,6]. Consequently, drug potency towards Na_V isoforms remains an open issue for tissue selectivity and efficacy/safety ratio. Moreover, preclinical studies showed that other antiarrhythmic drugs or their derivatives presented improved efficacy in myotonic conditions, compared to mexiletine[8,9,10].

The possibility to discriminate drug action between $Na_V1.4$ and cardiac $Na_V1.5$ is critical for exerting a therapeutic anti-myotonic effect, reducing the risk of cardiac toxicity. Efficient and reliable pre-clinical tests are pivotal, also with the long-term goal to find novel anti-myotonic agents. Furthermore, use of alternative drugs to safely treat myotonia could be pivotal for more efficient therapy. Our aim was to assess effects mexiletine and its derivative compound (VM11) both on $Na_V1.4$ and $Na_V1.5$ using an automated patch clamp platform.

2. Methods

Cell culture

We used TE671 cells (provided by Cell Line Services: https://cls.shop/), which endogenously express $Na_V1.4$, and CHO cells heterologously expressing $Na_V1.5$ (provided by Charles River: https://www.criver.com/find-cell-line).

Compounds

The compounds tested were 2-(2,6-dimethylphenoxy)-1-amino-propanemethylethylamine (Mex) and the mexiletine-derivative 2,2,5,5-tetramethyl-N-[1-methyl-2-(2,6-dimethylphenoxy)ethyl]-2,5-dihydro-1H (VM11). Stock solutions containing external solution and dimethyl sulfoxide (<0.33%) were used for both compounds.

Electrophysiology

Recordings of peak sodium currents (at – 20 mV) of $Na_V1.4$ and $Na_V1.5$ were performed using the automated patch clamp platform Patchliner. For dose response curves, at least 8 increasing concentrations were included. The tonic- (0.3 Hz), voltage- (-70 and -140 mV at 0.3 Hz) and use-dependent blocks (3 Hz for $Na_V1.5$ and 10 Hz for $Na_V1.4$), consistent with pathophysiological conditions of $Na_V1.4$ and $Na_V1.5$ by Mex/VM11, were evaluated.

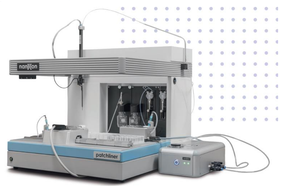

Figure 1. Patchliner: fully automated planar patch clamp system

3. Tonic (TB) and voltage-dependent (VDB) blocks

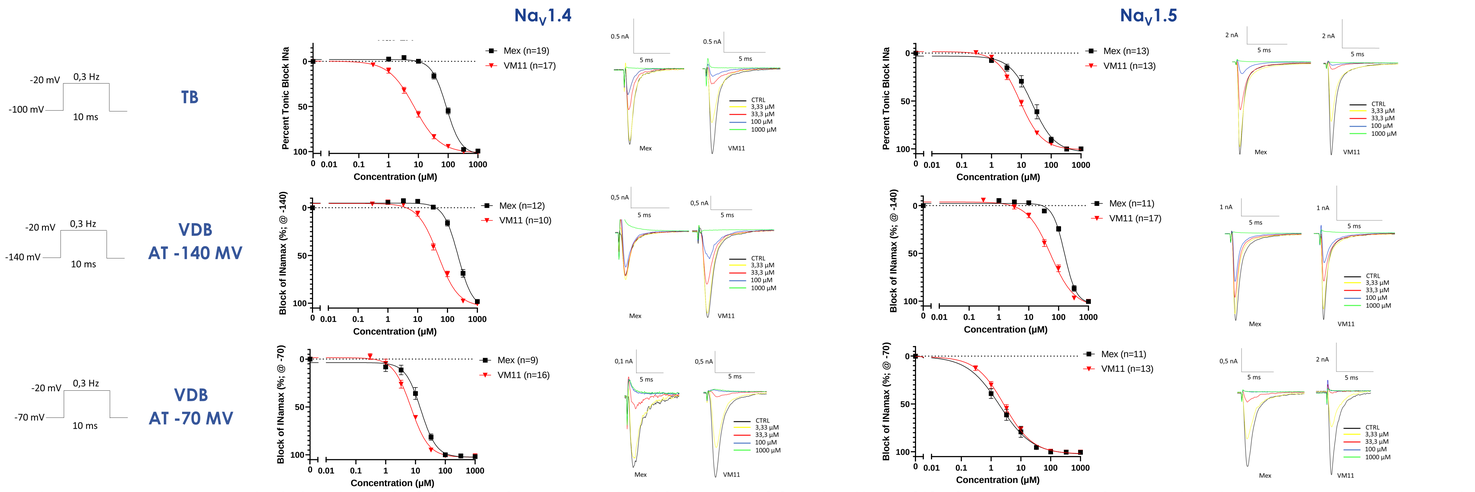

Figure 2: Dose-response curves depicting the tonic (A) and voltage-dependent block at -70 (B) and -140 mV (C) of Mex and VM11 for $Na_V1.4$ and 1.5. Mex was more potent in determining a TB of $Na_V1.5$ than of $Na_V1.4$ measured at 0.3 Hz (IC_{50}: 7 ± 0.6 vs 87 ± 5 µM). VDB at -140 mV showed that Mex was more potent in blocking $Na_V1.5$ than $Na_V1.4$. (159 ± 8 vs 226 ± 16 µM). On the other hand, VM11 produced similar TB and VDB (-140mV) of $Na_V1.4$ and $Na_V1.5$ (7 ± 0.6 vs 9 ± 0.5 µM for TB; 48 ± 3 vs 54 ± 5µM for VDB). Mex produced a more potent VDB (at -70mV) on $Na_V1.5$ than $Na_V1.4$ (2 ± 0.3 vs 15 ± 1µM). Similarly, VM11 blocked $Na_V1.5$ more than $Na_V1.4$ in VDB (at -70mV) conditions (3 ± 0.2 vs 7 ± 0.4 µM).

4. Use-dependent block (UDB)

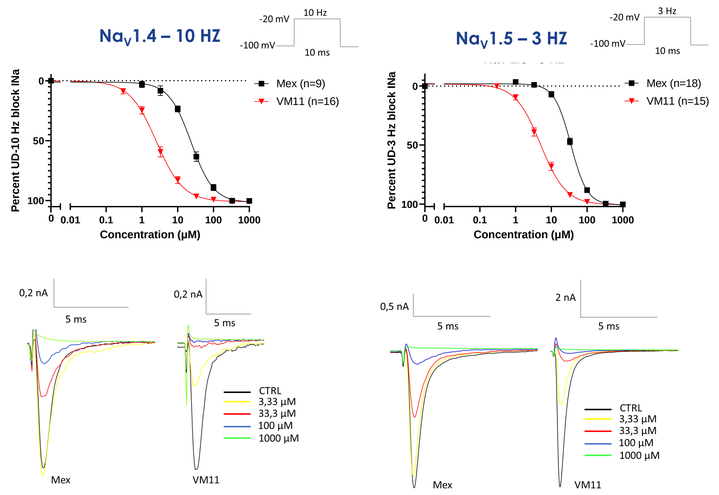

Figure 3. Dose-Response curves showing UDB of Mex and VM11 on $Na_V1.4$ and $Na_V1.5$. At cardiac pathophysiological frequency of 3 Hz, VM11 blocked $Na_V1.5$ more than Mex with an IC_{50} of 5 ± 0.3 µM (vs 35 ± 1 µM). At stimulation frequencies mimicking the myotonic discharge (10 Hz), both Mex and VM11 blocked $Na_V1.4$ channels with high potency (24 ± 2 and 3 ± 0.2 µM).

5.Conclusions

	Channel	IC_{50} (µM) Mex vs VM11
TB	1.4	87 ± 5 vs 7 ± 0.6
	1.5	24 ± 3 vs 9 ± 0.5
VDB @-140 MV	1.4	226 ± 16 vs 48 ± 3
	1.5	159 ± 8 vs 54 ± 5
VDB @-70 MV	1.4	15 ± 1 vs 7 ± 0.4
	1.5	2 ± 0.3 vs 3 ± 0.2
UDB - 10 Hz	1.4	24 ± 2 vs 3 ± 0.2
UDB - 3 Hz	1.5	35 ± 1 vs 5 ± 0.3

- ✓ Mex has more affinity for $Na_V1.5$ channel compared to $Na_V1.4$ in conditions of TB/VDB.
- ✓ VM11 was more potent in determining TB and VDB (at -140 mV) of $Na_V1.4$ than $Na_V1.5$, but blocks $Na_V1.5$ more than $Na_V1.4$ in condition of VDB at -70 mV.
- ✓ At pathological heart frequencies (3 Hz), VM11 blocked $Na_V1.5$ more than Mex, while at myotonic-like frequency of 10 Hz, Mex and VM11 potently blocked $Na_V1.4$ but still at concentrations in the range of blocking $Na_V1.5$.
- ✓ This biophysical approach can help the development of novel $Na_V1.4$ blockers with improved safety profile for muscle disorders.

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