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Increased sodium channel use-dependent inhibition by a new potent analogue of tocainide greatly enhances in vivo antimyotonic activity

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ABSTRACT

Although the sodium channel blocker, mexiletine, is the first choice drug in myotonia, some myotonic patients remain unsatisfied due to contraindications, lack of tolerability, or incomplete response. More therapeutic options are thus needed for myotonic patients, which require clinical trials based on solid preclinical data. In previous structure-activity relationship studies, we identified two newly-synthesized derivatives of tocainide, To040 and To042, with greatly enhanced potency and use-dependent behavior in inhibiting sodium currents in frog skeletal muscle fibers. The current study was performed to verify their potential as antimyotonic agents. Patch-clamp experiments show that both compounds, especially To042, are greatly more potent and use-dependent blockers of human skeletal muscle hNav1.4 channels compared to tocainide and mexiletine. Reduced effects on F1586C hNav1.4 mutant suggest that the compounds bind to the local anesthetic receptor, but that the increased hindrance and lipophilia of the N-substituent may further strengthen drug-receptor interaction and use-dependence. Compared to mexiletine, To042 was 120 times more potent to block hNav1.4 channels in a myotonia-like cellular condition and 100 times more potent to improve muscle stiffness in vivo in a previously-validated rat model of myotonia. To explore toxicological profile, To042 was tested on hERG potassium currents, motor coordination using rotarod, and C2C12 cell line for cytotoxicity. All these experiments suggest a satisfactory therapeutic index for To042. This study shows that, owing to a huge use-dependent block of sodium channels, To042 is a promising candidate drug for myotonia and possibly other membrane excitability disorders, warranting further preclinical and human studies.

1. Introduction

Blockers of voltage-gated sodium channels are clinically used in a number of disorders of plasma membrane excitability, including cardiac arrhythmias, epileptic seizures, pain, and myotonia (Imbrici et al., 2016). Many sodium channel blockers bind to the local anesthetics receptor located within the pore of the channel (Ragsdale et al., 1994, 1996). This receptor is highly conserved among sodium channel subtypes, thereby allowing most blockers to exert similar block of sodium channels expressed in central and peripheral neurons, cardiomyocytes and skeletal muscle fibers (England and de Groot, 2009). In most cases, the safety of these drugs relies on their ability to block sodium channels in a frequency-dependent manner, allowing a selective inhibition of over-excited cells while sparing the healthy organs. Recently, more selective blockers have been identified, especially for the peripheral nerve Nav1.7 and Nav1.8 subtypes, and some of them are being evaluated in clinical studies for the treatment of chronic pain (De Lera Ruiz and Kraus, 2015; Theile and Cummins, 2011).

Abbreviations: TRR, time of righting reflex; 9-AC, anthracene-9-carboxylic acid; SAR, structure-activity relationship.

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Myotonic syndromes are characterized by skeletal muscle stiffness due to sarcolemma hyper-excitability, which can be painful and badly interfere with daily motor activities and quality of life (Statland et al., 2014; Suetterlin et al., 2014). A randomized controlled clinical trial recently demonstrated the efficacy of the antiarrhythmic drug mexiletine in myotonia (Hoffman and Kaminski, 2012; Statland et al., 2012). Consequently, mexiletine received orphan drug designation for treatment of myotonic disorders by F.D.A. and E.M.A. (U.S. Food and Drug Administration, 2010; European Medicines Agency, 2013a; 2013b, 2014). By blocking skeletal muscle Nav1.4 sodium channels in a use-dependent manner, the drug inhibits the myotonic discharges of action potentials and favors muscle relaxation (De Luca et al., 1997; Desaphy et al., 1999). The drug also alleviates the transitory muscle weakness associated with recessive myotonia congenita due to chloride channel ClC-1 mutations (Lo Monaco et al., 2015). Nevertheless, a number of myotonic patients obtain little benefits from mexiletine due to contraindications, side effects, lack of tolerability, or lack of response (Matthews and Hanna, 2014; Suetterlin et al., 2015). In addition, mexiletine distribution has been interrupted by the manufacturer in a number of countries, making very difficult access to the drug. In sodium channel-related myotonias, unsatisfactory response to mexiletine may stem from a reduced affinity of the mutated channel to the drug, and other sodium channel blockers, like flecainide, may be successful in such cases (Desaphy et al., 2004, 2013c; 2016). The other orally-available lidocaine analogue, tocainide, has been also used in myotonia (Streib, 1986, 1987), but was associated with an elevated incidence of serious adverse reactions and discontinued in many countries. There is thus a general opinion that more therapeutic options are needed for myotonic patients, and that clinical trials based on solid preclinical data are urgently needed (Matthews and Hanna, 2014).

Searching for other useful antimyotonic drugs, we recently developed a pharmacologically-induced model of myotonia in the rat and investigated a number of marketed sodium channel blockers (Desaphy et al., 2013b, 2014). We found that the antimyotonic activity of drugs in vivo was closely parallel to the in vitro inhibition of sodium currents elicited by high-frequency voltage-clamp protocols in mammalian cells transfected with hNav1.4 cDNA. Beside the study of marketed drugs, we also explored the possibility to increase potency and use-dependent behavior through chemical optimization of mexiletine and tocainide in a series of SAR studies (De Bellis et al., 2013; De Luca et al., 2000, 2003a; 2003b, 2012). We have identified beta-proline derivatives of tocainide with a 10-fold increase in potency and use dependence for blockade of sodium channels in frog muscle fibers (Catalano et al., 2008). One of these compounds, namely NeP1 or To10 (Fig. 1), was 10 times more potent than tocainide in blocking human sodium channels and showed considerable analgesic activity in animal models of neuropathic pain (Ghelardini et al., 2010). Lately, on the basis of a 3D-QSAR study, new chemical maneuvers allowed to obtain two promising compounds 100 times more potent than tocainide, namely To040 and To042 (Carrieri et al., 2009; Muraglia et al., 2014) (Fig. 1).

The aim of the present study was to verify the potency of these two new compounds on the human Nav1.4 isoform expressed in mammalian cell line, obtain information about the molecular binding site, and test their antimyotonic activity in vivo in the rat model of myotonia (Desaphy et al., 2013b, 2014). Beside efficacy, we also performed experiments to explore the toxicological profile, including effects on hERG potassium channels and cytotoxicity. The results indicate To042 as a promising candidate drug for myotonia and, possibly, other plasma membrane excitability disorders, warranting further preclinical studies and hopefully clinical trials.

2. Materials and methods

2.1. Patch-clamp recording in HEK293 or tSA201 cell lines

Sodium or potassium currents were recorded using the whole-cell patch-clamp configuration in Human Embryonic Kidney 293 (HEK293) cells or tSA201 cells (a derivative of HEK293 cells) expressing the human skeletal muscle voltage-gated (hNav1.4) sodium channel or the human Ether-a-go-go Related Gene (hERG) potassium channel. Permanent transfection of HEK293 cells with wild-type hNav1.4 channel or its F1586C mutant has been previously described (Desaphy et al., 2009, 2012). Transient transfection of tSA201 cells with 8 μg of pcDNA3.1 expression vector containing the coding region of hERG channel (a generous gift from prof. Valeria Casavola, University of Bari, Italy; originally from prof. E. Ficker, MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH) and 1 μg of a vector encoding the CD8 gene reporter was performed using the calcium-phosphate co-precipitation method (Desaphy et al., 2001). Only cells decorated with micro-beads coated with anti-CD8 antibody were used for patch-clamp 24 at 48 h after transfection.

Patch-clamp recordings were performed at ambient temperature (20–22 °C) using an Axopatch 1D amplifier (Axon Instruments, Molecular Devices, Sunnyvale, CA). Voltage clamp protocols and data acquisition were obtained with pClamp 10.2 software (Axon Instr.), through a 12-bit A-D/D-A interface (Digidata 1440, Axon Instr.). Pipettes were fabricated from Corning 7052 glass capillaries (Garner Glass, Claremont, CA, USA) using a vertical PP-82 puller (Narishige, Tokyo, Japan), to obtain tip resistance in between 1.5 and 3.5 MΩ. Currents were low-pass filtered at 2 kHz (−3 dB) and digitized at 10–20 kHz. Five minutes after establishing the whole-cell configuration, sodium or potassium currents were recorded using specific voltage-clamp protocols described in the Results section. Data were analyzed off-line using Clampfit 10.2 and SigmaPlot 10.0 (Systat Software GmbH, Erkrath, Germany).

2.2. In vivo evaluation of the antimyotonic activity of To042

Animal housing and experiments were performed in accordance with the Italian Guidelines for the use of laboratory animals, which conforms to the European Union Directive for the protection of experimental animals (2011/63/EU), and received approval from the Animal Experimentation Ethic Committee of the University of Bari (CESA prot. 7/12) and Italian Health Department (Decreto n. 91/2013-B). All efforts were made to minimize animal suffering and to reduce the number of animals used. Twelve adult Wistar rats (body weight: 350–500 g; Charles River Laboratories, Calco, Italy) were individually housed and received water and food ad libitum. Experiments were performed in 3–4 rats simultaneously as previously described (Desaphy et al., 2013b, 2014). Briefly, myotonia condition was induced in the rats by i.p. injection of 30 mg/kg body weight of 9-anthracene-carboxylic acid (9-AC), a potent blocker of skeletal muscle ClC-1 chloride channels. A few minutes after 9-AC injection, animals constantly show a manifest muscular stiffness and difficulties to move, with no noticeable side effects (Desaphy et al., 2013b). The myotonia was quantified by measuring the time of righting reflex (TRR), that is time needed by the rat to turn back on its four limbs from the supine position. Ten minutes after 9-AC injection, the rats were administrated an oral dose of drug (mexiletine or To042) or vehicle saline solution using an esophageal cannula. The TRR was determined 10 min before 9-AC injection and 30, 60, 120 and 180 min after 9-AC. At each time point, the TRR value was calculated as the mean of 7 determinations, repeated at 1-min intervals to prevent the warm-up phenomenon. For each drug dose, the experiments were repeated at least 3 times in different rats, and experimental data are given as the mean ± S.E.M.