Biochimica et Biophysica Acta 1838 (2014) 1693–1700

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Biochemical characterization of sporadic/familial hemiplegic migraine mutations

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ARTICLE INFO

Article history:
Received 15 November 2013
Received in revised form 26 March 2014
Accepted 27 March 2014
Available online 3 April 2014

Keywords:
Na^+,K^+-ATPase
ATP1A2
Familial hemiplegic migraine
FHM2
Alpha 2 isoform
Baculovirus expression system

ABSTRACT

Sporadic hemiplegic migraine type 2 (SHM2) and familial hemiplegic migraine type 2 (FHM2) are rare forms of hemiplegic migraine caused by mutations in the Na^+,K^+-ATPase α2 gene. Today, more than 70 different mutations have been linked to SHM2/FHM2, randomly dispersed over the gene. For many of these mutations, functional studies have not been performed. Here, we report the functional characterization of nine SHM2/FHM2 linked mutants that were produced in *Spodoptera frugiperda* (Sf)9 insect cells. We determined ouabain binding characteristics, apparent Na^+ and K^+ affinities, and maximum ATPase activity. Whereas membranes containing T345A, R834Q or R879W possessed ATPase activity significantly higher than control membranes, P796S, M829R, R834X, del 935–940 ins Ile, R937P and D999H membranes showed significant loss of ATPase activity compared to wild type enzyme. Further analysis revealed that T345A and R879W showed no changes for any of the parameters tested, whereas mutant R834Q possessed significantly decreased Na^+ and increased K^+ apparent affinities as well as decreased ATPase activity and ouabain binding. We hypothesize that the majority of the mutations studied here influence interdomain interactions by affecting formation of hydrogen bond networks or interference with the C-terminal ion pathway necessary for catalytic activity of Na^+,K^+-ATPase, resulting in decreased functionality of astrocytes at the synaptic cleft expressing these mutants.

1. Introduction

The Na^+,K^+-ATPase is a cation-transporting membrane protein involved in active transport of sodium and potassium ions against their concentration gradients across the cell membrane. For every molecule of ATP consumed, three sodium ions are exported from the cell in exchange for import of two potassium ions. The Na^+,K^+-ATPase serves many roles, including cell-volume homeostasis as well as maintenance of the membrane potential. In addition, the generated Na^+ and K^+ gradients drive various secondary active transporters. Since transport of Na^+ and K^+ across the cell membrane takes place against such steep concentration gradients, the enzyme consumes high amounts of energy: approximately 25–50% of cellular ATP is consumed by the Na^+,K^+-ATPase [1,2].

The Na^+,K^+-ATPase consists of at least two different subunits: an α-subunit (110 kDa), containing ten transmembrane α-helices (αM1–αM10) and two large cytosolic loops (αM2–αM3 and αM4–αM5), together with a highly glycosylated single transmembrane domain spanning β-subunit (50 kDa). In some tissues, a third subunit containing a characteristic FXYD motif is present as well (γ-subunit) [3]. In the human genome, four α-subunit encoding genes have been described. The proteins encoded by these genes share a sequence homology of approximately 80–90% and are expressed tissue specifically [4]. For example, the α1-isoform is expressed ubiquitously [5], whereas the α4-isoform is detected only in the testis [6]. The α3-isoform is present in both the central and peripheral nervous systems [7,8], whereas the α2-isoform is expressed in different tissues including heart skeletal muscle, vascular smooth muscle, bone, adipocytes, and astrocytes [9].

Genetic studies have linked over 70 mutations in the *ATP1A2* gene (chromosome 1q23) encoding the α2-isoform of the Na^+,K^+-ATPase to sporadic/familial hemiplegic migraine type 2 (SHM2/FHM2), shown in Fig. 1 [10]. Sporadic (only one affected family member) and familial (two or more affected family members) hemiplegic migraine are autosomal-dominant monogenic subtypes of migraine with aura (MA), characterized by neurological aura symptoms preceding the migraine attack. In addition to the mutations present in the α2-isoform of Na^+,K^+-ATPase, two other genes have been linked to FHM: *CACNA1A* has been linked to SHM1/FHM1 whereas *SCN1A* has been associated with FHM3 (no sporadic mutations reported) [11]. Together, SHM and FHM show a prevalence of approximately 0.005% [12,13].

The exact pathophysiology of SHM2/FHM2 remains to be elucidated, but it is assumed that α2-isoform Na^+,K^+-ATPase mutations result in

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http://dx.doi.org/10.1016/j.bbamem.2014.03.022
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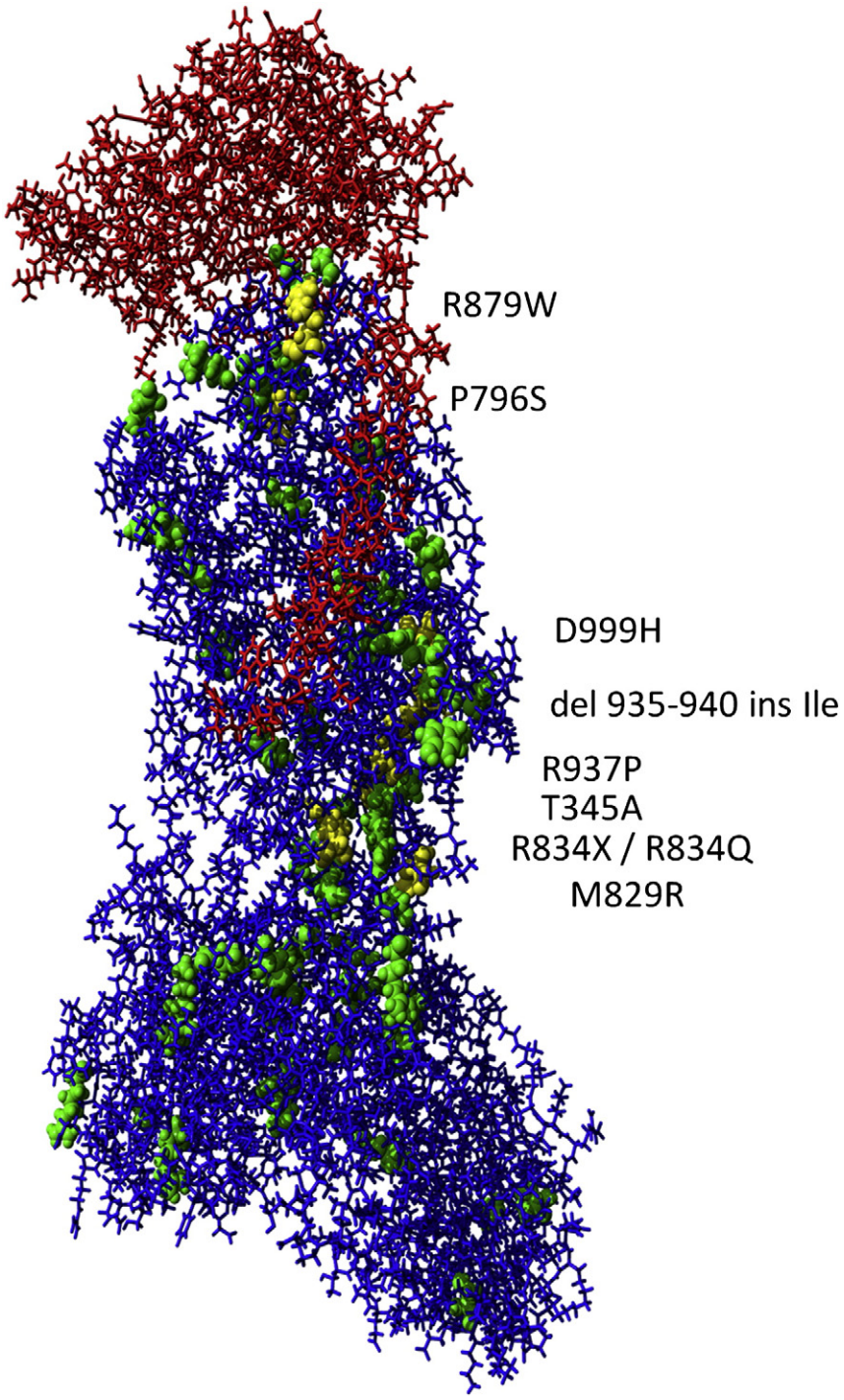

Fig. 1. Homology model of the α2-isoform of Na^+,K^+-ATPase (based on PDB ID: 2ZXE [38]) showing the residues that are associated with SHM2/FHM2 mutations in literature highlighted in green, whereas the mutations described in the present study are highlighted in yellow. The α-subunit is shown in blue, whereas red represents the β-subunit: these two subunits together constitute a functional Na^+,K^+-ATPase.

altered enzyme functionality at the synaptic cleft, where astrocytes expressing the α2-isoform are present as supporting cells. The Na^+, K^+-ATPase present at the plasma membrane of these cells presumably plays a role in uptake of K^+ from the synaptic cleft following nerve excitation, resulting in a favorable driving force for the re-uptake of glutamate by the co-localized glutamate transporter [14]. Changes in the enzymatic properties of the Na^+,K^+-ATPase might therefore have an indirect effect on signal transmission via the synaptic cleft, leading to hemiplegic migraine attacks.

Over 70 SHM2/FHM2 causing mutations in the *ATP1A2* gene have been reported, randomly dispersed over the gene [15]. For many of these mutations the effects on enzyme functionality have not been reported yet, whereas investigation of the biochemical effects of each of these mutations on protein functionality is an essential step towards understanding the pathogenesis of SHM2/FHM2 mutations. Using *Spodoptera frugiperda* (Sf)9 insect cells lacking endogenous Na^+,K^+-ATPase, we set out to characterize the enzymatic effects of nine SHM2/FHM2 mutations (all except T345A) located within the