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Review

What can naturally occurring mutations tell us about $Ca_v1.x$ channel function?☆

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ABSTRACT

Voltage-gated Ca^{2+} channels allow for Ca^{2+}-dependent intracellular signaling by directly mediating Ca^{2+} ion influx, by physical coupling to intracellular Ca^{2+} release channels or functional coupling to other ion channels such as Ca^{2+} activated potassium channels. L-type Ca^{2+} channels that comprise the family of Ca_v1 channels are expressed in many electrically excitable tissues and are characterized by their unique sensitivity to dihydropyridines. In this issue, we summarize genetic defects in L-type Ca^{2+} channels and analyze their role in human diseases (Ca^{2+} channelopathies); e.g. mutations in $Ca_v1.2$ α1 cause Timothy and Brugada syndrome, mutations in $Ca_v1.3$ α1 are linked to sinoatrial node dysfunction and deafness while mutations in $Ca_v1.4$ α1 are associated with X-linked retinal disorders such as an incomplete form of congenital stationary night blindness. Herein, we also put the mutations underlying the channel's dysfunction into the structural context of the pore-forming α1 subunit. This analysis highlights the importance of combining functional data with structural analysis to gain a deeper understanding for the disease pathophysiology as well as for physiological channel function. This article is part of a Special Issue entitled: Calcium channels.

Contents

1. Introduction

Voltage-gated calcium channels (VGCCs) govern important physiological functions such as contraction, secretion, neurotransmission, and gene expression in many different cell types by mediating Ca^{2+} entry into electrically excitable cells in response to membrane depolarization [1]. VGCCs, like other ion channels, do not operate as isolated proteins, but instead form signaling complexes with signaling molecules, receptors, other types of ion channels [2,3]. A central pore-forming α1-subunit determines most of the channel's biophysical and pharmacological properties. In the cell membrane, the calcium channels form a hetero-oligomeric complex with auxiliary β- and α2δ-subunits, and in some cases δ-subunits. VGCCs can be divided into the group of L-type calcium channels (LTCCs, Ca_v1 family) and non-LTCCs (Ca_v2 and Ca_v3 family). We will focus in this review on LTCCs.

The LTCCs comprise the isoforms $Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$ and $Ca_v1.4$. Their functional diversity reaches from excitation–contraction coupling in muscle, to stimulus secretion coupling in sensory and endocrine cells, cardiac pace-making, as well as neuronal firing to learning and memory [1]. They can be distinguished pharmacologically from other VGCCs by their high sensitivity towards organic Ca^{2+} channel blockers and activators. Among those, dihydropyridines (DHPs) proved especially important because of their very high binding affinity, their high selectivity for LTCCs and their diversity, being both Ca^{2+} channel blockers (e.g. isradipine, nifedipine) and Ca^{2+} channel activators (e.g. BayK 8644) [4,5]. The Ca^{2+} channel blockers in clinical use (as such nifedipine, amlodipine, verapamil and diltiazem) have mainly cardiovascular effects as they preferentially block $Ca_v1.2$ channels in the cardiovascular tissue.

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The $Ca_v1.3$ channels are expressed together with $Ca_v1.2$ in several tissues including sinoatrial nodes, heart atria, neurons, chromaffin cells and pancreatic islets. In particular, $Ca_v1.3$ serves an important role in the sinoatrial node [6] and in chromaffin cells [7], and they shape neuronal function [8] and help to support pace-making in vulnerable dopaminergic substantia nigra neurons [9]. Importantly the use of DHPs has been shown to be associated with a decreased incidence of Parkinson's disease in humans [10]. In the hippocampus $Ca_v1.2$ mediates long-term potentiation, spatial learning and memory [11], whereas $Ca_v1.3$ mediates long-term potentiation in the amygdala and participates in the consolidation of fear memory [12].

$Ca_v1.4$ channels are predominantly expressed in retinal cells where they play an important role, as evident from mutations in the voltage-gated calcium channel gene *CACNA1F* encoding $Ca_v1.4$ LTCCs that cause several forms of retinal diseases in humans (OMIM: 300071, 300476, 300600). $Ca_v1.4$ knock-out mice support this view as these mice show severe visual deficiencies [13,14]. $Ca_v1.4$ expression is also reported in dorsal root ganglia neurons [15], mast cells [16] and T-lymphocytes [17].

$Ca_v1.1$ channels possess a more restricted expression pattern. This channel is expressed almost exclusively in skeletal muscle cells. Conformational changes of voltage-sensing domains in $Ca_v1.1$ are transmitted by a mechanical linkage to the associated ryanodine receptors that release Ca^{2+} from the sarcoplasmic reticulum [1].

All VGCCs are capable of sensing intracellular Ca^{2+} levels. This is very likely a safety mechanism to protect cells from calcium overload via activity-dependent feedback mechanisms such as Ca^{2+}-dependent inactivation (CDI), mediated by C-terminally bound calmodulin [18,19]. Mechanisms that inhibit CDI play an important role in sensory cells (as such for $Ca_v1.4$ in retinal cells and for $Ca_v1.3$ in auditory cells, see [20]) allowing the inducement of graded and tonic presynaptic depolarization and making neurotransmitter release dependent on sustained activation of presynaptic LTCCs.

Diseases caused by mutations in genes encoding ion channel subunits or their regulatory proteins are referred to as 'channelopathies'. A large number of distinct channel dysfunctions have been described to be caused by mutations in the channel's 1α subunits. Mutations in LTCCs may result in a loss-of-function, in which the LTCC mediated Ca^{2+} influx is (much) reduced or completely abolished whereas gain-of-function mutations confer new or enhanced activity. However, apparently such enhanced activity has an unwarranted positive connotation. Gain-of-function mutations may result in enhanced Ca^{2+} influx, but this increased sensitivity does not necessarily result in improved signaling. Instead, this might result in a loss-of-control of existing Ca^{2+} signaling pathways.

Here we summarize the role of selected LTCCs in human diseases that are caused by genetic defects in these Ca^{2+} channels ('Ca^{2+} channelopathies') and we discuss the functional effects of the structural aberrations within the pore-forming α1 subunit that underlie the channel's dysfunction. Table 1 summarizes $Ca_v1.2$, $Ca_v1.3$ and $Ca_v1.3$ α1 subunit related diseases. Mutations in the $Ca_v1.1$ channel, leading to hypokalemic periodic paralysis and malignant hyperthermia sensitivity are reviewed elsewhere [21]. We elaborate on common structural 'hotspots' that result in either loss- or gain-of-channel function in all the three LTCCs and discuss them in the structural context of a $Ca_v1.4$ channel homology model (Fig. 1 and Table 1). The model of the $Ca_v1.4$ channel was created using the structure of NavAB (PDB ID: 3RVY, [22]) as a template. The sequences of human $Ca_v1.x$ and NavAB were first aligned with MUSCLE [23], then models of the $Ca_v1.4$ channel were created using MODELLER version 9.8 [24]. The best model based on the DOPE score was selected for analysis [25].

2. $Ca_v1.2$-related channelopathies

$Ca_v1.2$ is the predominantly expressed LTCC in the cardiovascular system. Its dysfunction can cause severe cardiac diseases in humans often associated with sudden cardiac death. Patients carrying mutations in the *CACNA1C* gene – that encodes for the $Ca_v1.2$ channel – may suffer from Timothy syndrome (TS, [26,27]), Brugada syndrome (BS, [28]), and in some cases are diagnosed together with shorter as normal QT intervals (sQT; [29]) and early repolarization syndrome (ERS, [29]). Among those, TS is a multiorgan disease; the patients may suffer from syndactyly (fusion of fingers and/or toes), immune deficiency, intermittent hypoglycemia, cognitive abnormalities and autism [26,27] in addition to cardiac arrhythmias that are often associated with sudden death. $Ca_v1.2$ mutations from patients with BS so far functionally analyzed led to a loss-of-function (Tables 1 and 2) showing a still controversially discussed [28–30] loss-of-trafficking phenotype. In contrary, two mutations in patients suffering from TS show a $Ca_v1.2$ channel gain-of-function [26,27]. The ventricular arrhythmias caused by these mutations are severe and the majority of TS patients seldom survived beyond the age of three years. The two mutations were initially identified as de novo mutations. However, they may actually also represent parental mosaicism [27,31]. The low number of reports in the literature of patients/families carrying $Ca_v1.2$ (and also $Ca_v1.3$) mutations (Table 1) might be due to mild(er) phenotypes that could eventually escape detection in case of somatic mosaicism.

The gain-of-function of a glycine-to-serine mutation at position 402 (Gly402Ser) at the cytoplasmic end of segment IS6 (Fig. 1) resulted in a strong reduction of voltage-dependent inactivation (VDI, [26]). A similar effect that included a slight shift in the voltage-dependence of activation was observed for a glycine-to-arginine substitution just four amino acids downstream at position 406 (Gly406Arg) in helix 6. The change of channel function was irrespective of the co-expressed β-subunit [27,32]. Importantly the same mutation at position 406 can be located in exon 8A [VNDAV-coding exon] where it results in a relatively mild phenotype of Timothy syndrome (named TS1 in [26]) compared to its occurrence in the alternative exon 8 (MQDAM-coding exon). The latter more severe variant named TS2 [27] can be rationalized by the higher expression of exon 8 in the heart and brain (80% versus 20% exon 8A [27]). Neither heterozygous nor homozygous TS2-like mice were viable. Most likely the predominant expression of exon 8 in the brain and heart resulted in a lethally high level of mutated channels [33]. However, heterozygous mice that still carried the inverted neomycin cassette in exon 8A (TS2-neo) survived through adulthood. It is unclear, whether or not heterozygous TS2-neo mice better tolerated the mutation because the neo-cassette lowered expression levels of the mutated channel; supporting biochemical data are missing. Interestingly behavioral phenotyping showed that TS2-neo mice have normal general health and activity but show a markedly restricted, repetitive and preservative behavior, altered social behavior, altered ultrasonical vocalization as well as enhanced tone-cued and contextual memory following fear conditioning despite displaying normal anxiety levels [33]. These data suggest that these mice also show autism-related behavior corresponding to core aspects of autism and autism spectrum disorders seen in humans.

Kinetic models suggested that the functional consequences of an almost complete loss of VDI of $Ca_v1.2$ channels in cardiac cells results in a prolongation of action potential duration and in increased calcium transients [34]. This correlates well with the observed prolongation in the QT interval in the affected individuals. Indeed such effects are evident from Ca^{2+} signaling experiments in Timothy syndrome cardiomyocytes derived from human skin cells of Timothy syndrome patients that were reprogrammed and induced to produce pluripotent stem cells. Mutation Gly406Arg led to significantly larger and prolonged Ca^{2+} elevations suggesting that channel inactivation is important for maintaining timing and amplitude of the ventricular Ca^{2+} release [35]. Similar effects were also observed in iPSC-derived neuronal cells on action potential widening [36]. Thus, drugs that directly interfere with the $Ca_v1.2$ inactivation gating mechanism are likely to improve cardiac arrhythmias as well as other severe symptoms affecting TS patients. Yarotskyy and colleagues [37]