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Review

Regulation of T-type calcium channels: Signalling pathways and functional implications

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ABSTRACT

T-type calcium channels (T-channels) contribute to a wide variety of physiological functions, especially in the cardiovascular and nervous systems. Recent studies using knock-out mouse models have been instrumental in documenting further the role of T-channels in sleep, heartbeat, pain and epilepsy. Importantly, several novel aspects of the regulation of these channels have been identified over the last few years, providing new insights into their physiological and pathophysiological roles. Here, we review recent evidence supporting that the Ca_v3 subunits of T-channels are modulated by endogenous ligands such as anandamide, zinc, redox and oxidizing agents, as well as G-protein and protein kinases pathways. The study of T-channel mutations associated with childhood absence epilepsy has also revealed new aspects of Ca_v3 subunit trafficking. Collectively, these findings identify novel regulatory mechanisms involved in the fine tuning of T-channel expression and activity, and offer new directions for the design of novel therapeutic strategies targeting these channels.

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1. Introduction

For almost two decades, until their cloning in the late 99s, T-type calcium channels (T-channels) were considered as an electrophysiological curiosity, being activated and open close to the membrane's resting potential (also designated LVA for low-voltage activated) in most excitable cells, especially in neurons [1,2]. The presence of T-type calcium currents (T-current) in a wide variety of cells, as well as in early development and disease models, resulted in many hypotheses regarding the role of T-channels being made. However, validating them has proven problematic due to the lack of selective T-channel blockers. The identification of three genes: CACNA1G, CACNA1H and CACNA1I, coding for the T-channels $Ca_v3.1/\alpha_{1G}$, $Ca_v3.2/\alpha_{1H}$ and $Ca_v3.3/\alpha_{1I}$, respectively (see Table 1; reviewed in [3–5]), was a significant landmark in the field, offering novel technical approaches to tackle the properties and significance of T-channels in physiology and pathology [6,7]. These last 10 years, therefore, many aspects have been identified, validating former observations and providing novel exciting paradigms regarding the properties and roles of T-channels.

2. T-type calcium channels: molecular basis of their diversity

Three genes code for T-channels (Table 1) and analysis of the various corresponding cDNAs clearly indicate that many splice variants exists for the three $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ subunits. Of importance, the cloned Ca_v3 subunits express well in heterologous systems (*Xenopus* oocytes, mammalian cells…), which allows detailed electrophysiological studies of the $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ subunits. The three recombinant Ca_v3 subunits generate T-type/low-voltage activated calcium currents (T-current) with distinct biophysical properties [8] and the $Ca_v3.1$ and $Ca_v3.2$ subunits generate T-currents that are highly reminiscent to those recorded in native cells (neurons, cardiac cells…). As mentioned above, alternative splicing notably enhances the diversity of T-channel isoforms [9–11] and there is growing evidence for significant differences in the electrophysiological properties of these splice variants of a given Ca_v3 subunit, especially the $Ca_v3.1$ subunit isoforms [5,12,13]. Overall, this bulk of data clearly supports that the molecular and functional diversity of T-channels in native cells can primarily arise from the expression of a wide variety of splice variants of the three Ca_v3 subunits. Interestingly, one should note that it is almost exclusively the intracellular regions of the Ca_v3 subunits that are subject to alternative splicing, especially the loops between the transmembrane domains and the C-terminal region [5]. It is therefore tempting to hypothesize that the modulation of T-channels, including second messenger pathways, is also impacted by alternative splicing, although this has yet to be experimentally supported.

Because they are phylogenetically related, T-channels should be compared to other voltage-activated calcium channels, i.e. the L- N- P/Q- and R-types that belong to the high-voltage-activated (HVA) family. One important feature of HVA channels is their heteromultimeric

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Table 1
T-type calcium channel genes and related diseases

Channel type	Gene name	Chromosome loc.	SNPs/mutations in human diseases	Ref.
$Ca_v3.1$	CACNA1G	17q22	Juvenile myoclonic epilepsy (JME)	[50]
$Ca_v3.2$	CACNA1H	16p13.3	Childhood absence epilepsy (CAE) and other idiopathic generalized epilepsies (IGE)	[47,49]
			Autism spectrum disorder (ASD)	[56]
$Ca_v3.3$	CACNA1I	22q13	–	

The three genes encoding the Ca_v3 subunits of T-type calcium channels, as well as their chromosome localization, are listed here. Additional information regarding these genes can be found elsewhere, especially at OMIM or HUGO websites (http://www.ncbi.nlm.nih.gov/omim/ and http://www.genenames.org/). T-channels are susceptibility genes for epilepsy. Many variants for CACNA1H are found in epileptic patients, especially for CAE (see text).

composition, with the β, $\alpha_2\delta$ and γ ancillary subunits playing prominent targeting and biophysical roles in the channel's activity [14,15]. To date, evidence for an interaction between HVA ancillary subunits and T-channels is sparse but data supporting a regulatory role for some of the β, $\alpha_2\delta$ and γ subunits of HVA channels have been reported [16,17]. Because Ca_v3 channels display properties fairly well matching those of native T-channels, it seems reasonable to speculate that T-channel auxiliary subunit(s), if any, would play a more discrete functional role compared to that of the β, $\alpha_2\delta$ and γ subunits of HVA channels. Purification and biochemical characterization of HVA channels had also benefited from the existence of selective ligands (i.e. dihydropyridines and toxins), as well as a considerably large panel of subunit-specific antibodies. This is not yet the case for the Ca_v3 subunits, preventing, until now, the characterization of T-channel complexes from native tissues [18].

3. New insights into the modulation of T-type calcium channels

Modulation of Ca_v3/T-channels has only recently been substantiated. We previously reported that the extensive study of T-channel modulation in native tissues did not provide support for the existence of generic modulation pathways of T-channels [19]. Using functional expression of recombinant Ca_v3 channels, many recent studies have now validated the idea that T-channels can be modulated by various endogenous ligands as well as by second messenger pathways. One of the first descriptions of T-channels being regulated by an endogenous ligand was made with the endocannabinoid anandamide [20], a bioactive lipid also known to be active on CB receptors, TRPV1 channels and two-pore K^+ channels [21]. Anandamide, as well as arachidonic acid, other N-acyl ethanolamides (AEA) and polyunsaturated fatty acids (PUFA) [22,23], inhibit Ca_v3 channels in the micromolar range through a membrane-delimited – possibly direct – interaction. Among the other signalling molecules that potently affect T-channels, zinc has been focused on. Zinc somehow acts as a neurotransmitter as it is released from the presynaptic vesicles of glutamatergic neurons (sometimes described as “gluzinergic” neurons) and free zinc modulates many membrane receptors, transporters and channels [24]. Interestingly zinc differentially regulates the three Ca_v3 channel isotypes: it preferentially inhibits $Ca_v3.2$ channels with an IC_{50} in the submicromolar range (~0.8 μM), which is 100 and 200-fold lower than what is observed for $Ca_v3.1$ and $Ca_v3.3$ channels, respectively [25]. In addition, zinc significantly slows the deactivation kinetics of the $Ca_v3.3$/T-current, i.e. the tail current, which consequently causes an enhanced calcium entry through $Ca_v3.3$ channels [25]. In these experimental conditions, it appears that zinc operates as a mixed blocker/opener of $Ca_v3.3$ channels. Interestingly, Todorovic et al. recently reported that the reducing agent L-cysteine, which can upregulate T-current in nociceptive neurons [26], likely produces its effect through chelation of free zinc ions that mediate a tonic block of $Ca_v3.2$ channels [27]. Zinc produces its blocking effect on $Ca_v3.2$ channels through binding to an extracellular histidine (His191) residue localized in the S3–S4 segment of domain I of $Ca_v3.2$ channel [27]. His191 was originally identified as a critical determinant of the nickel block of $Ca_v3.2$ channels [28]. In addition, the oxidizing agent, ascorbate, also produces $Ca_v3.2$ channel inhibition through the metal-catalyzed oxidation of this specific His191 [29], further demonstrating that this amino-acid is an important checkpoint in the modulation of $Ca_v3.2$ channel activity (Fig. 1).

Many G-protein coupled receptors (GPCRs) are potential modulators of T-channels in a large variety of cells types [19] and several aspects of GPCR modulation of Ca_v3 channels have recently been documented. Using a heterologous system, it was shown that the activation of the muscarinic M1 receptor could result in a selective G protein-induced inhibition of $Ca_v3.3$ channels through a $G\alpha q/\alpha 11$ pathway [30]. By contrast, activation of corticotropin releasing factor receptor 1 (CRFR1), which selectively inhibits $Ca_v3.2$ channels, likely mediates its effect through a cholera toxin sensitive, Gβγ-dependent pathway [31]. Notably, it was shown in a previous study that $Ca_v3.2$ inhibition by the G protein β_2 and γ ($G\beta_2\gamma$) subunits can occur via a direct interaction between the $G\beta_2\gamma$ subunits and the channel's intracellular linker between domains II and III (II–III loop) [32,33]. Several other recent studies have shown that Ca_v3/T-channels can be also modulated by second messengers and protein kinase pathways. Welsby et al. provided evidence that CamKII selectively promotes the activation of $Ca_v3.2$ channels [34]. A series of $Ca_v3.1$/$Ca_v3.2$ chimera was used to pinpoint the role of the II–III loop of $Ca_v3.2$ and, ultimately, the Serine 1198 residue was found to be instrumental in the CamKII regulation of $Ca_v3.2$ channels as it is dynamically phosphorylated in vivo [35]. Other Serine/threonine kinases as well as protein kinases A and C (PKA and PKC) have also been shown to enhance activity of the three recombinant Ca_v3 subunits of T-channels, both in *Xenopus* oocytes [36,37] and in mammalian cells at physiological temperature [38]. The specific residue(s) involved in these serine/threonine kinase modulations have not been identified yet, although they are known to require the II–III loop, both for $Ca_v3.2$ in the case of PKA [37] and for $Ca_v3.1$ in the case of PKC modulation [36]. By contrast, activation of Rho kinase/ROCK via lysophosphatidic (LPA) receptor activation results in an inhibition of $Ca_v3.1$ and $Ca_v3.3$ currents and involves two clusters of serine and threonine residues of the II–III loop of $Ca_v3.1$ [39]. Overall, this wealth of data strikingly identifies the intracellular II–III loop of Ca_v3 channels as an important determinant for channel

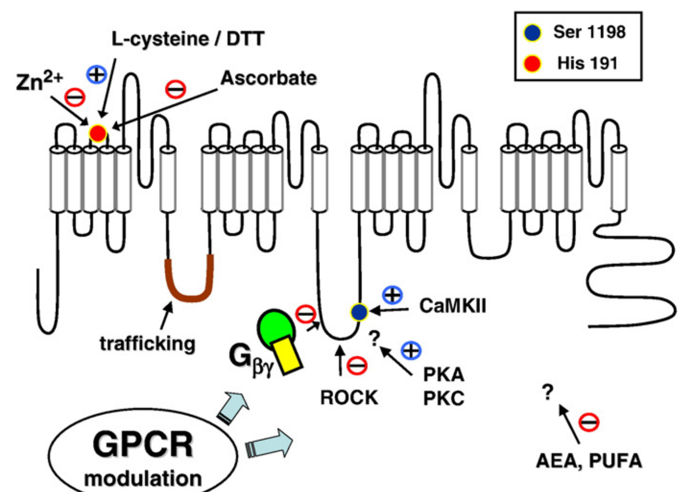

Fig. 1. A schematic representation of the recently identified regulatory pathways for Ca_v3 channels, especially for $Ca_v3.2$ channels (see text for details and references).

regulation by serine/threonine kinases and G-protein pathways (Fig. 1). Forthcoming studies should aim at further identifying the relevance of these recently described mechanisms converging at the II–III loop locus, reminiscent of a crosstalk mechanism identified for Ca_v2 channels [40], as well as evaluating whether or not the other intracellular domains of Ca_v3 channels play a role in the GPCR-mediated regulation of Ca_v3 channels. Through these upcoming studies, many of the "orphan" modulations of native T-channels, such as an ATP-dependent potentiation of T-channels in thalamocortical neurons [41,42], should be puzzled out.

4. T-type calcium channels and related channelopathies

4.1. Childhood absence epilepsy (CAE)

T-channels play an important role in neuron excitability, especially in the thalamocortical loop, and therefore are considered as susceptibility genes in epilepsy. The description of an increase in T-channel activity in thalamic neurons of animal models of absence epilepsy was provided a long time ago with the GAERS rat [43], as well as more recently using the mouse lines *tottering*, *lethargic*, *stargazer* and *coloboma* [44,45] and the WAG/rij rat [46]. In humans, mutations in the CACNA1H ($Ca_v3.2$) and CACNA1G ($Ca_v3.1$) genes have been identified in epilepsy patients [47–50] (see Table 1). Originally, Chen et al. [47] found twelve single nucleotide polymorphisms (SNPs)/missense mutations in CACNA1H associated with childhood absence epilepsy (CAE). Heron et al. [48,49] have extended the number of SNPs in CACNA1H linked to idiopathic generalized epilepsy (IGE) and proposed that these variants contribute to patients' susceptibility to epilepsy but are not alone sufficient to cause generalized epilepsy. Electrophysiological characterization of the corresponding $Ca_v3.1$ and $Ca_v3.2$ mutants in heterologous expression systems has revealed only discrete biophysical alterations that are unlike to support a significant gain of channel activity [50–52]. Looking at the distribution of the CAE mutations in the $Ca_v3.2$ protein, it appears that many of these mutations are present in the linker between domains I and II (I–II loop) of the protein. This suggests a functional role of this intracellular region. Indeed, the I–II loop regulates expression of the $Ca_v3.2$ subunit at the plasma membrane [53]. While $Ca_v3.2$ channels are markedly retained in intracellular compartments (only ~12% at the plasma membrane), it appears that deletion in the I–II loop significantly enhances surface expression of $Ca_v3.2$ channels: +300% [53]. This is in contrast with a higher surface expression of both $Ca_v3.1$ (~25%) and $Ca_v3.3$ channels (~30%), in which deletion in the I–II loop produces mild changes in surface expression (+40% and −30%, respectively) [54]. The determinants involved in $Ca_v3.2$ trafficking are preferentially contained in the central region of the I–II loop. Along with these data, it was shown that all the CAE mutants of $Ca_v3.2$ exhibit significant increase in surface expression [53]. Finally these experiments also provide evidence that the three Ca_v3 subunits have in common a proximal domain of the I–II loop – adjacent to the domain I – that contributes to the gating properties, which has been described as a gating brake [54,55]. Altogether, these studies indicate that a more comprehensive view of the structure–function relationships of Ca_v3 channels can be gained from the study of disease-related mutations.

4.2. Autism spectrum disorder (ASD)

Recently, several missense mutations linked to autism spectrum disorder (ASD) were found within CACNA1H [56]. All these mutations significantly altered $Ca_v3.2$ channel activity, causing a positive shift in activation properties and a reduction in conductance. Mutations in $Ca_v3.2$ channels may not be the only impairment causing the ASD phenotype: autism is a complex, behaviourally defined, static disorder of the immature brain. Mutations in CACNA1H may act as modifiers of its phenotypic expression. Interestingly, it should be stressed that one third of ASD patients suffer from epilepsy [56]. Based on the critical role of T-channels in neuronal development and excitability [57], it is reasonable to consider that mutations in CACNA1H may affect proper neuronal function, especially during neurogenesis [58].

5. Modulation of T-type calcium channel expression: more than a disease marker?

Analysis of the density of T-currents strongly suggests that their expression is highly modulated during development as well as in several experimental pathological situations [57]. Only a few examples will be reviewed here. In a rat model of post myocardial infarction, de novo expression of T-channels is observed in ventricular myocytes [59] and is reminiscent of the embryonic pattern of Ca_v3 subunit expression in heart [60]. In addition, angiotensin II and endothelin-1 are able to induce ventricular hypertrophy and T-channel expression [61,62]. Similarly, up-regulation of T-channel expression occurs in several neurological diseases. Along with the description linking T-channels and genetic absence epilepsy (see above), there is evidence for an increase in T-channel expression in CA1 pyramidal neurons in a rat model of temporal lobe epilepsy [63]. Also, along with recent data indicating that T-channels play a crucial role in tuning sensory neuron excitability and serve as amplifiers in peripheral pain transmission [64,65], Jagodic et al. [66] have described an upregulation of T-channel expression in a subpopulation of DRG sensory neurons in rats with painful diabetic neuropathy.

An important challenge will now be to resolve whether de novo, or up-regulation, of T-channel expression is adaptative or maladaptative of these various disease states. Regarding the implication of T-channels in the cardiac hypertrophy phenotype: T-channel activity could represent a deleterious calcium entry mechanism affecting calcium handling and contractile properties in hypertrophied cardiac myocytes. This is partly supported by pharmacological studies showing that hypertrophy can be attenuated in the presence of T-channel blockers [67]. Conversely, it may be worth considering that calcium entry through T-channels, which plays an important role in developing cardiac myocytes, could contribute to counterbalance the deleterious effect of hypertrophy. Indeed, a recent study revealed that mice overexpressing $Ca_v3.1$ channels in the heart, under the control of the α-myosin heavy chain promoter, did not develop a pathological cardiac phenotype, i.e. hypertrophy or arrhythmogenic sudden death [68]. This data, which also indicates that $Ca_v3.1$ T-channels are ineffective in triggering excitation–contraction coupling, suggests that overexpression of T-channels does not induce cardiac dysfunction. Additional experiments are required to further identify the role of T-channels in hypertrophic or post-infarction remodelled heart. The recent development of knock-out animals for $Ca_v3.1$ and $Ca_v3.2$ may offer new opportunities to address these important questions. Similar hypotheses and objectives can be formulated regarding the up-regulation of T-channels in CA1 and DRG neurons in temporal lobe epilepsy and diabetic neuropathy, respectively.

Because T-channels are present in many transformed cell lines, such as neuroblastoma, retinoblastoma, glioma cells and thyroid carcinoma cells, it is hypothesized that a link exists between these channels and cancer [67]. Interestingly it appears that in one given tissue, T-channel expression can be modulated in opposite ways in cancer cells. The $Ca_v3.2$ subunit of T-channels is overexpressed in neuroendocrine prostate LNCaP cells [69], as well as in primary cells obtained after surgical removal of prostate cancer biopsies [70]. An increase in $Ca_v3.2$ activity may account for enhanced autocrine/paracrine secretions in neuroendocrine prostate cancer, i.e. prostatic acid phosphatase (PAP) synthesis and secretion [70]. In contrast, the gene coding for $Ca_v3.1$ is hypermethylated in several cancer types,

especially in colorectal cancer [71], leading to a down-regulation of $Ca_v3.1$ expression.

6. Pharmacology of T-type calcium channels

For years the "ion channel community" has been waiting for selective blockers for T-channels and it seems that this recent post-cloning era is full of promises. Many reviews have described various organic molecules, members of many drug classes: dihydropyridines, succinimide derivatives, diphenylbutylpiperidine derivatives, benzodiazepines, anesthetics … that are currently used to treat a variety of neuronal and cardiovascular diseases and are inhibitors of T-channels. Unfortunately, most of these classical T-channel blockers do not allow discrimination between T-channels and other VGCC-related signals and none are selective enough for T-channels [72–74]. There is a great need for selective blockers in the T-channel toolkit as many new hypotheses regarding the pathophysiological role of T-channels are emerging. One can now anticipate that a new generation of more selective T-channel blockers will soon be available [75–77] and, hopefully, these new T-channel blockers may also have appeal in clinical use as they may hold therapeutic interest in treating severe pain and epilepsy [77] and possibly lowering heart rate and decreasing blood pressure.

7. Perspectives and conclusion

Many new insights into T-channel properties and roles have been gained this last decade. Ten years after the cloning of the Ca_v3 subunits, knock out animals for $Ca_v3.1$ and $Ca_v3.2$ have been produced and will be helpful to further explore the involvement of T-channels in a wide variety of physiological and pathophysiological states. In $Ca_v3.1-/-$ mice that show no significant histological and physiological abnormalities, the complete lack of T-currents in thalamocortical relay neurons [78] and in sinoatrial node cells [79] has further documented the major role of T-channels in neuronal burst firing and cardiac pacemaker activity. In $Ca_v3.2-/-$ mice, the role of this channel isoform in nociception was confirmed [65]. Interestingly, $Ca_v3.2-/-$ mice show significant vasoconstriction of coronary arteries. This phenotype may be related to altered endothelial nitric oxide (NO)-mediated vasodilation that is observed in $Ca_v3.2-/-$ arteries, and/or to altered activity of the large conductance calcium-activated potassium (BK_{Ca}) channels that were found associated to the $Ca_v3.2$ protein [80]. Knock out mice are undoubtedly useful animal models to probe the physiological and pathophysiological roles of T-channels. However, constitutive inactivation of these genes in animals may lead to compensatory phenomenons that mask the precise involvement of either $Ca_v3.1$ or $Ca_v3.2$ activity. For example, discrepancies in the neuropathic pain phenotype between $Ca_v3.2-/-$ mice and animals that undergo antisense knock-down [64,65] suggest that compensatory effects in knock-out animals alleviate their response to hyperalgesia. Unfortunately, no report of the functional consequences of $Ca_v3.3$ knock-out has been published to date.

Are T-channels multimeric complexes? Besides data regarding interaction of the $Ca_v3.2$ protein with the BK_{Ca} channel [80], G-protein $\beta_2\gamma_2$ subunits [32], as well as the attempts to identify whether the HVA auxiliary subunits physically associate with Ca_v3 subunits [16,17], it is expected that Ca_v3 proteins form larger complexes with currently unknown partners. In the coming years, such partners should be identified.

Are T-channels relevant drug targets? Recent efforts have led to the identification of more selective T-channel blockers [77] and it is expected that T-channel antagonists are potential therapeutic agents, especially for the treatment of neurological diseases [72–74]. With the recent identification of isoform-specific properties, the need to design ligands selective for a given T-channel subtype has emerged. The challenge will then be to define whether specific T-channel isoforms/variants can be selectively targeted for therapeutic intervention.

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