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AMAZING T-TYPE CALCIUM CHANNELS: UPDATING FUNCTIONAL PROPERTIES IN HEALTH AND DISEASE

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Abstract
T-type Ca^{2+} channels have gained 15 years after cloning an immense interest as novel players in very unexpected cell functions and many relations to diseases have been discovered. This Special Issue provides a state of the art overview on novel functional properties of T-type Ca^{2+} channels, unexpected cellular functions and most importantly will also summarizes and review the involvement of this "tiny, transient" type of Ca^{2+} channels in several disease. It is tried to bridge the gap between molecular biophysical properties of T-type Ca^{2+} channels and diseases providing finally a translational view on this amazing ion channel.

Keywords: voltage-gated ion channels/ calcium channels/ modulation/ recruitment/ antagonist/ animal models/ disease/

Running title: *Low-voltage-activated T-type Ca^{2+} channels: an overview*

The advent of T-type Ca^{2+} channels

Calcium ions function as the most widespread, versatile and promiscuous signalling molecule within intracellular cell compartment. Changes in the concentration of free cytosolic Ca^{2+} ($[Ca^{2+}]_i$) are of fundamental importance in gene expression, different stages of the cell cycle, starting from the fertilization and embryonic pattern formation over cell differentiation and proliferation to cell death and cell necrosis. Modulation of $[Ca^{2+}]_i$ is essential for different cellular processes such as gene transcription, transmitter release, muscle contraction, secretion, learning and memory (3). It is known since the early 1950th that a major part of these changes in $[Ca^{2+}]_i$ depend on a Ca^{2+} influx via the plasma membrane (see for an concise overview 33). However, the first Ca^{2+} currents, recorded with a double sucrose gap voltage clamp device, were described only on 1967 (39). It became clear, especially after refining the electrophysiological methods (19, 22) that Ca^{2+} permeates through highly selective Ca^{2+} channels. The accepted paradigm was that probably only "a" Ca^{2+} channel existed, although a first indication of the presence of a heterogeneity of Ca^{2+} channel populations was obtained already in 1975 (18) using a two-electrode voltage clamp method in starfish eggs. However, the first clear evidence for functionally different highly Ca^{2+} selective channels came in 1984–85. The so called, low-voltage-activated (LVA) or T-type (from "tiny" and "transient") Ca^{2+} channels were directly measured as single channel events by using the patch-clamp technique in sensory neurones (6, 34) and ventricular myocytes (32). Fast inactivating burst-like openings were detected at very low voltages in excised membrane patches of sensory neurons with activation-inactivation kinetics and holding sensitivity very similar to that of macroscopic low-threshold currents (see figure 2 in 6). The clear-cut separation between high-voltage activated (HVA) or L-type (from "large" and "long-lasting") and T-type channels in single channel measurements, occurred soon after and came as a surprise. If Ba^{2+} was the charge carrier, long-lasting openings representing the ~25 pS L-type channel could be easily resolved even in the same patch from burst-like openings representing T-type Ca^{2+} channels of ~7 pS (see e.g. figure 3 in 32). The tiny single channel conductance with both Ba^{2+} or Ca^{2+} being the charge carrier through T-type channel was later confirmed an many other studies (8, 13). In this period, it was also evident that besides activating transiently at very negative membrane potentials (2, 6, 14), the neuronal T-type channel possessed two other unique key properties: a very slow deactivation rate (1, 5) and an effective recruitment of full size low-threshold Ca^{2+} currents following short hyperpolarization (7, 11). Both features, could nicely account for the existence of Ca^{2+}-mediated rebound spikes observed in the inferior olive and thalamic neurons (25-27) , named "low-threshold Ca^{2+} spikes" (see for a recent review 23).. This feature was also described at the single channel level in cardiac myocytes (13). Interestingly, some unexpected single channel properties, e.g. the voltage dependence of the first latency for single channel openings, could be nicely translated into the macroscopic voltage dependent inactivation of T-type Ca^{2+} channels (13).

In the same period (1985-89), the canonical biophysical properties of neuronal and cardiac T-type channels were established (2, 4, 8, 13, 14, 16) and it was also clearly anticipated that possibly several genes were responsible for encoding different types of Ca^{2+} channels. Now, we know that 10 genes encode for the pore forming α_1-subunit of

different Ca^{2+} selective ion channels (α1S, C, D, F, A, B, E and for T-type α1G, H, I). In addition, we also know that 10 genes for non-pore forming Ca^{2+} channel subunits exist (α2δ1-4, β1-4, γ1, 6) which modulate the functional properties and the expression pattern of Ca^{2+} channels, but have not been proven so far to play a role for T-type channels (for the most recent review see 20).

T-type Ca^{2+} channels: molecular identity and selective antagonists

Surprisingly, it took more than 10 years before the three T-type Ca^{2+} channel genes were identified (29, 35-37). Three α1-subunits were cloned (α1G, α1H and α1I or Cav3.1, Cav3.2 and Cav3.3), which were further extended by alternative splicing (36). This was a decisive step to allow the unraveling of structural determinants for permeation and kinetic T-type channel properties (41, 44). Even more exciting, the functional role of T-type Ca^{2+} channels could now be studied systematically, the distribution and expression pattern of the three T-type isoforms in several tissues could be unraveled, and, importantly, opened the possibility to use antisense and knock-out strategies to unveil the physiological and pathological role of these channel (for some reviews from the early period after cloning see 15, 33, 35, 36, 42, 43). The molecular cloning of the T-type Ca^{2+} channels is now approximately 15 years old and has been so far the focus of the new developments in this exciting field.

Amazingly enough, it took even longer (24 years!) to develop a new generation of selective T–type channel blockers (40) that could overcome the weak specificity of anti-hypertensive drugs (mibefradil) (9, 30) and divalent cations (Ni^{2+}) toward T-type channels. Mibefradil and Ni^{2+} have been for years the only pharmacological tools on hands of physiologists and pharmacologists to dissect the effects of T-type from other voltage-gated channels. Their usage, however, was often limited to a qualitative identification of T-type channel expression for specific cellular functions, particularly when Ca^{2+} currents could not be directly measured. It is not surprising, therefore that the recent synthesis of piperidine derivatives (TTA-P2) (40), which potently and selectively block T-type channels (12), boosted the number of reports on T-type channels in the last 5 years and allowed the discovery of unexpected cell functions in which low-voltage activated channels are involved (21, 28).

T-type channels are now recognized as therapeutic targets in a variety of diseases, like epilepsy, insomnia, pain, cancer and hypertension (for recent reviews see 38). It can thus be predicted that present and future Ca^{2+} channel antagonists selective for Cav3 channels will be increasingly used to better understand the role of these channels in the regulation of key body functions with a search for clinical products.

A Special Issue on T-type Ca^{2+} channels: amazing new functional roles

The first ideas about a functional impact of T-type Ca^{2+} channels came from heart muscle and central neurons rhythmicity. In one case, the T-type channel has been hypothesized to play a critical role in cardiac pacemaking (17, 31). In the other case, T-type channel was essential for the synchronized repetitive activity of thalamic neurons (10, 25)

characteristic of certain stages of sleep (see for an historical overview 24). Later, T-type channels were considered as important for smooth muscle contraction, hormone and neurotransmitter secretion, gamete interaction and gene expression (discussed in a historical overview on T-type channel in 33). Research in the last 10 years has indicated a plethora of new cell functions in which T-type Ca^{2+} channels are involved.

This special issue of Pflügers Archiv focuses on the multiple roles that T-type channels play in the regulation of critical body functions. T-type channels control neuronal excitability, muscle contraction, hormone and neurotransmitter release, pain sensation, cell development and proliferation. Thus, it is not surprising that up- or down-regulation or critical mutations of Cav3 channels can cause neurodegenerative diseases (epilepsy, sleep disorders, stress, hyperalgesia), as well as tumor growth and cardiovascular diseases (cardiac arrhythmias, heart block and hypertension). In this special issue we have therefore given equal emphasis to the new functional roles of T-type channels and to the search of newly available T-type channel antagonists with potential use in the therapy.

In the first article related to the structure and function of Cav3.1, Cav3.2 and Cav3.3 channels Dr Edward Perez-Reyes discusses the importance of two critical regions of the channel: the extracellular high affinity metal binding site and the intracellular loop connecting repeats I and II (the "gating brake") which stabilizes Cav3 channel gating and regulates surface channel expression. Dr. Yaroslav Shuba then describes the mechanism by which Ca^{2+} ions permeate through open Cav3 channels and how the intrapore binding sites for Ca^{2+} of LVA channels differs from that of HVA channels. Along these lines, Dr. David Spafford compares the impressive similar structure and function of Cav3 channels of invertebrates and mammals providing insights into key shared features between these distant channels.

In relation to the interaction of T-type channels with other ion channels, membrane proteins or bioactive lipids, Drs. Ray Turner and Gerald Zamponi focus on the newly discovered property of T-type to form signaling complexes with different calcium-gated (KCa1.1 and KCa3.1) and even with voltage-gated K^+ channels via associated KChIP proteins, broadening the regulatory role of Cav3 channels in neuronal excitability. Dr. Emilio Carbone discuss new emerging views on the role of T-type channels in controlling synaptic transmission in central neurons and vesicular exocytosis in neuroendocrine cells in the light of recent reports on the co-localization of T-type channels with ion channels, membrane receptors and SNARE proteins. Drs. Jeanne Chemin and Philippe Lory describe the inhibitory effects of various endogenous lipids on Cav3.2 and Cav3.3 channels and how these modulatory effects could be critically associated with pain sensation and vasodilation.

Concerning the involvement of T-type channels in neurodegenerative diseases, Dr. Slobodan Todorovic provides a detailed overview of the role that T-type channels play in the increased sensitization of pain response in animal models of type 1 and type 2 peripheral diabetic neuropathy. Dr. Emmanuel Bourinet discusses the role of T-type in the perception and modulation of pain and how recent T-type channel blockers could be possibly used as new analgesic. Dr. Hee-Sup Shin focuses on recent studies on the T-type channels in absence epilepsy showing that T-type channels of excitatory thalamocortical neurons rather than T-type channels of the inhibitory thalamic reticular nuclei are crucial for the generation of spikes and wave discharges at the origin of the disease. On the same

topic, Drs. Régis Lambert, Nathalie Leresche and Vincenzo Crunelli discuss how the use of selective T-type channel blockers helps understanding the key function of T-type "window current" in the control of slow waves and spindles of natural sleep in thalamic neurons. Dr. Chung-Chin Kuo provides new emerging evidence on the role of T-type window current in the genesis of bursts discharges in neurons of subthalamic nucleus of parkinsonian models and how T-type channel antagonists could be beneficial for the treatment of Parkinson's disease. In relation to the neuroprotective action of T-type channel blockers, Dr. Janxin Bao describes in details the efficacy of newly developed chemical compounds with promising neuroprotective effects.

Regarding the T-type channels expressed in the cardiovascular system, Dr. Cary Hill discusses the peculiar properties of Cav3 channels and their coupling to Ca^{2+}-gated K^{+} channels in smooth muscle tissues and how their rapid up- or down-regulation can affect peripheral vasoconstriction. In line with this, Dr. Christopher Fry furnishes a detailed description of T-type channels expressed in the muscle and epithelial tissues of the urinary and male genital tracts and how an up-regulation of their activity could induce functional disorders (prostate tumors). Dr. Matteo Mangoni describes the relevance of T-type channels in impulse conduction and heart rate automaticity of sino-atrial and atrio-ventricular nodes using mice models, discussing their significance in the light of recent studies on the role of T-type channels in congenital heart block in humans.

Concerning the specific involvement of T-type channels in cancer, Dr. Jaroslav Dziegielewski discusses recent findings which identify the T-type channels as the molecular target for anticancer therapy and indicate new directions for the design of novel therapeutic strategies using T-type channel antagonists. Along this line, Dr Jin Tao focuses on the use of T-type channel blockers for the treatment of highly proliferative tumors like glioblastoma. Drs. Alberto Darzson and Arturo Hernandez-Cruz complete the issue providing a detailed description of the evidence for the role of Cav3 channels in spermatogenic cells and in spermatozoa

Obviously, this reviews collection of world's leading T-type channel researchers does not cover all the many exciting aspects on the structure and function of Cav3 channels. It certainly provides a topical overview of pertinent issues that will be further developed in future studies, hopefully with the entry of new research groups.

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