A Tale of Two (Calcium) Channels

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Calcium influx through voltage-dependent calcium channels triggers excitation-contraction coupling and regulates pacemaking activity in the heart. Two distinct families of calcium channels have been identified in cardiac tissue: L-type calcium channels, which are essential in triggering Ca$^{2+}$ release from internal stores, and low-voltage-activated (LVA) T-type calcium channels (ICaT), whose role remains obscure in physiological and pathophysiological conditions. Functional features of ICaT include low threshold of activation, small unitary conductance, slow activation, and fast inactivation inducing a typical crisscrossing pattern of current traces for increasing depolarizations, negative steady-state inactivation, and slow deactivation kinetics. In addition, T-type currents compared with L-type currents are more sensitive to block by mibebradil and Ni$^{2+}$ ions. Whereas L-type calcium channels have been extensively characterized at the functional and molecular levels, classical cloning strategies failed to identify an α1 subunit encoding for a T-type channel.

An alternative approach to identifying new members of the calcium channel family used in silico cloning strategies with a search of genetic databases for sequences homologous but not identical to known Ca$^{2+}$ channel α1 subunits. The identification of several expressed sequence tags and genomic sequences corresponding to a subset of distantly related α1 subunits resulted in the identification of full-length cDNAs encoding three distinct α1 subunits: α1G in rat, mouse, and human, and α1H in human and rat. Because α1H was obtained by screening a human heart library, it was originally considered the cardiac T-type channel isoform because of the presence in Northern blot analysis of a strong signal for α1H in the heart. However, an α1G transcript is also detected in adult heart, and it has become an interesting challenge to identify which isoforms underlie ICaT in cardiac cells.

The article by Satin and Cribbs in this issue of Circulation Research aims to identify the α1 isoform encoding the ICaT1 in AT-1 cells, an immortalized cell line derived from mouse atrial tissue. The advantages of using this cell line after a short period of culture are the absence of sodium current and the relatively small amplitude of L-type current, allowing for a better isolation of ICaT. Satin and Cribbs have compared the properties of ICaT to those of the recombinant calcium currents generated by α1G and α1H expressed in human embryonic kidney cells. Most of the investigations are devoted to a comparison of the biophysical properties between ICaT from AT-1 cells and α1G/α1H currents. The results indicate that most basic electrophysiological properties, such as current-voltage relationship and activation, inactivation, and deactivation properties, are rather similar and not discriminative. However, the recovery from inactivation of T-type currents, characterized by the sum of a fast and a slow time constant, is described as a functional signature of T-type channel isoform expression. The major difference between the two T-type channel isoforms relates to the relative amplitude of the slow recovery rate ($\tau_s$) as a strong functional criterion to establish a linkage between ICaT from AT-1 cells and α1G current. Such linkage is confirmed by reverse transcription–polymerase chain reaction (RT-PCR) experiments from cultured AT-1 cells using primers designed to amplify the III-IV loop of the three isoforms. The results show that only α1G, and predominantly a specific variant thereof, is revealed from sequencing of the subcloned PCR product corresponding to a single major band.

The results presented on AT-1 cells are convincing, but it is reasonable to wonder whether they can be extended to any cardiac cell. Comparing the properties of native ICaT in cardiovascular cells with those of recombinant currents may be difficult because many studies have been conducted either under different experimental conditions or in different species. Cardiac T-type currents have often been studied in cultured embryonic or neonatal myocytes because of their low expression (or absence) in adult atrial (or ventricular) myocytes, except in several species such as chicken and guinea pig. Other studies report the characterization of cardiac ICaT induced by hormone treatment 9 or patholog 10,11. On the other hand, T-type channel isoforms have also been cloned from different species, and the use of various external concentrations of calcium or barium ions to study their related currents sometimes confounds the comparisons between native and recombinant currents. Satin and Cribbs 8 provide a comparison between the properties of the recombinant rat (α1G) and human (α1H) currents and ICaT from AT-1 (mouse) cells in a physiological calcium concentration. However, a large number of splice variants have been described for α1G in the different species 9 and as discussed by the authors, it cannot be ruled out that splice variations in regions other than the III-IV loop may influence biophysical properties including recovery from inactivation. All of these splice variations involve connecting loops or the C-terminus.

Curiously, nickel sensitivity was not investigated by Satin and Cribbs, even though it is considered an important assay to distinguish between the two isoforms because of the much higher Ni$^{2+}$ sensitivity of α1H currents (IC_{50} 5 μmol/L)
versus α1G currents (IC_{T1}>150 \mu mol/L).\textsuperscript{12,3} Preliminary data revealed a low Ni\textsuperscript{2+} sensitivity (160 \mu mol/L) of cardiac T-currents in freshly dissociated atrial myocytes from neonatal rat cells, also suggesting a linkage with the α1G isoform.\textsuperscript{13} However, previous data in rabbit sinoatrial,\textsuperscript{14} adult guinea pig cells,\textsuperscript{15} and rat hypertrophic cells\textsuperscript{11} indicate that T-currents are totally blocked by a lower concentration of Ni\textsuperscript{2+} ions (about 40 \mu mol/L). This would suggest either a differential expression of α1G and α1H isoforms among cardiac tissues, interspecies differences, or a possible developmental switch between isoforms. The latter hypothesis appears consistent with the results of Monteil et al,\textsuperscript{3} who observed in a dot-blot analysis a developmental regulation of human α1G transcripts with a prominent signal in embryonic compared with the adult heart. It must be emphasized, in agreement with such a hypothesis, that no IC_{T1} has yet been recorded in adult human heart.\textsuperscript{16} Another study worth mentioning used an antisense strategy, which suggested that the cardiac T-type current in 3-week-old rats is related to the α1E subunit.\textsuperscript{17} This result remains puzzling because some properties such as threshold of activation and deactivation properties differ markedly between IC_{T1} and α1E currents. In addition, IC_{T1} from freshly dissociated neonatal rat atrial tissue was found\textsuperscript{13} to be insensitive to the α1E-specific toxin SNX 482. It is, however, worthwhile to note that T-type channel expression was induced by growth hormone treatment in the experiments of Piedras-Renteria et al.\textsuperscript{17}

By contrast, with the absence of T-type current in adult human heart, an LVA calcium current was first reported in human atria,\textsuperscript{18} sharing some typical properties with sodium channels such as TTX sensitivity (designated IC_{TTX}). Its description in rat\textsuperscript{19} and guinea pig\textsuperscript{20} myocytes indicated that it is not exclusively observed in diseased human cells. The study of Heubach et al\textsuperscript{21} in this issue of Circulation Research demonstrates for the first time the coexistence of IC_{TTX} with IC_{T1} in guinea pig ventricular myocytes. The results show that adult rat myocytes lack IC_{T1} whereas adult guinea pig myocytes express both LVA currents that can be isolated by pharmacological dissection using TTX and Ni\textsuperscript{2+} in the absence of external sodium ions. As mentioned above, T-type currents are reported here to be blocked by 40 \mu mol/L Ni\textsuperscript{2+} ions (IC_{50} 16 \mu mol/L), a concentration much lower than that required to block recombinant α1G currents but rather close to that reported to block α1H currents. There are major differences between the biophysical properties of IC_{T1} and IC_{TTX}. IC_{TTX} exhibits a run-up after the rupture of the patch membrane, a lower voltage for peak current (10 mV), a more negative steady-state inactivation relationship, faster time constants for recovery from inactivation, and a faster rate of deactivation. Interestingly, IC_{TTX} was blocked by mibefradil, as is IC_{T1}.

The remaining question concerns the molecular basis of IC_{TTX}. Is this current related to modified sodium channels in the absence of external sodium ions\textsuperscript{20} or is it due to a new population of channels? Answering this will require the molecular identification of the pore-forming subunit using various strategies, including antisense, expression cloning, and other conventional molecular and biochemical isolation techniques. There is a lack of evidence for a correlation between IC_{TTX} and the controversial slip-mode conductance that has been described for sodium channels in the presence of protein kinase A (PKA) and cardiotonic steroids.\textsuperscript{22} The fact that IC_{TTX} does not require activation of the PKA pathway argues for a distinct population of channels. Additional experiments to test the effect of PKA inhibitors on IC_{TTX} would be of interest. Heubach et al\textsuperscript{21} underline some discrepancies between the TTX concentration required to block modified sodium channels and IC_{TTX}. In addition, the work of Lemaire et al\textsuperscript{18} on human atrial cells showed that IC_{TTX} similarly conducts calcium and barium ions. Permeation to barium ions was not tested on the modified sodium channels, but most of the conclusions were based on calcium transient measurements. This property seems atypical for sodium channels but might indicate either a strong alteration of the sodium channel permeability in the absence of extracellular sodium ions or molecular similarities between IC_{T1} and IC_{TTX}.

From the results presented in this issue of Circulation Research and other studies, it is now clear that two LVA channels (IC_{T1} and IC_{TTX}) can coexist in cardiac myocytes. What is the physiological role of these LVA channels in the heart? T-type channels are assumed to play a role in pacemaking activity because of their presence in sinoatrial node and the negative chronotropic effect of Ni\textsuperscript{2+} ions.\textsuperscript{14} Whether IC_{TTX} is also expressed in sinoatrial cells is unknown, and it would also seem important to reinvestigate the role of LVA versus L-type calcium channels in the pacemaking activity. An interesting feature of T-type calcium currents is related to their slow deactivation kinetics and the existence of a window current in the range of the cell membrane resting potential. Slow deactivation can mediate larger calcium influx than high-voltage-activated channels during short depolarizations, as shown by the application of a neuronal-type action potential such as a voltage-clamp command on recombinant α1G channels,\textsuperscript{3} the calcium transients in response to different spike shapes or frequencies also being isoform dependent.\textsuperscript{23} In spite of their rapid kinetics of inactivation, the existence of a window current would confer a role of T-type channels in maintaining intracellular Ca\textsuperscript{2+} concentration. T-type currents do not seem to play an important role in cardiac excitation-contraction coupling,\textsuperscript{24} but they could contribute to fine-tuning of basal calcium levels and control physiological processes such as hormone secretion, as previously suggested in adrenal cells.\textsuperscript{25} Their expression is also cell cycle dependent\textsuperscript{26} and their involvement in cell growth and proliferation was suggested in cardiomyocytes and smooth muscle cells,\textsuperscript{27,28} respectively. In contrast to IC_{T1}, IC_{TTX} is observed in human heart and is likely to be sensitive to mibefradil as found in guinea pig myocytes.

Future studies will probably look for an overexpression of IC_{T1} and IC_{TTX} in pathological conditions, which would be expected to generate cardiac arrhythmias. Interestingly, it was recently reported that mibefradil prevents tachycardia-induced electrophysiological remodeling in dogs.\textsuperscript{29} Thus, inhibition of both LVA currents by mibefradil might be considered in terms of pathophysiology. Combining specific functional assays and molecular tools should allow a better...
understanding of the physiological and pathological roles of LVA channel isoforms in cardiovascular cells.

References


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