Identification of a T-Type Ca\textsuperscript{2+} Channel Isoform in Murine Atrial Myocytes (AT-1 Cells)

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Abstract—Calcium channels are important targets for therapeutics, but their molecular diversity complicates characterization of these channels in native heart cells. In this study, we identify a new splice variant of a low-voltage activated, or T-type Ca\textsuperscript{2+}, channel in murine atrial myocytes. To date, a1G and a1H are the only 2 T-type Ca\textsuperscript{2+} channel isoforms found in cardiovascular tissue. We compared a1G and a1H channel current heterologously expressed in HEK 293 cells with T-type current from the murine atrial tumor cell, AT-1. AT-1 cell T-type current (I\textsubscript{T}) has the same voltage dependence of activation and inactivation as a1G and a1H. The cloned T-type channels and AT-1 T-type current share similar kinetics of macroscopic inactivation and deactivation. The kinetics of recovery from inactivation of T-type currents serves as an electrophysiological signature for T-channel isoform. a1G and AT-1 I\textsubscript{T} have a similar recovery from inactivation time course that is faster than that for a1H. In all cases, T-type current recovers with a biexponential time course, and the relative amplitude of fast and slow time courses explains the slower a1H recovery kinetics, rather than differences in the time constants of the individual transitions. Thus, the T-type channels may be an important contributor to automaticity in heart cells, and molecular diversity is reflected in the pathway of recovery from inactivation. (Circ Res. 2000;86:636-642.)

Key Words: Ca\textsuperscript{2+} channel ■ patch-clamp ■ electrophysiology ■ gating ■ atrial

Low-voltage activated (LVA) Ca\textsuperscript{2+} channels differ from high-voltage activated channels on the basis of their low-voltage activation range, slow deactivation, equivalent single-channel conductance of Ca\textsuperscript{2+} or Ba, and Na\textsuperscript{+} channel-like macroscopic kinetic pattern (reviewed in Reference 1). Similar to Na\textsuperscript{+} channels, the time to peak and the macroscopic inactivation of T-type channel current (I\textsubscript{T}) become faster with increasing depolarization. This results in the signature pattern of current traces elicited by increasing depolarizations crossing over when superimposed. The recently cloned a1G, a1H, and a1I channels exhibit these unique generalized T-type channel functional characteristics in heterologous expression systems.\textsuperscript{2-4} However, detailed comparisons of the cloned channel properties versus T-type Ca\textsuperscript{2+} channels in native cardiac systems have not been reported.

An important consideration for evaluating functional parameters of T-type channel current is that it is difficult to clearly isolate I\textsubscript{T} in native cells such as cardiac myocytes. T-type Ca\textsuperscript{2+} current recorded from native tissue is often either a subtraction current or recorded in the absence of Na\textsuperscript{+}. Subtraction of currents elicited after depolarized holding potentials from more hyperpolarized holding potentials is commonly used to separate T- and L-type Ca\textsuperscript{2+} current. Studies that measure T-type current in the presence of Na\textsuperscript{+} (reviewed in Reference 5) have the more difficult task of separating overlapping Na\textsuperscript{+} channel and Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange current. In neurons, tetrodotoxin (TTX) can be used to eliminate Na\textsuperscript{+} current. However, TTX blockade of Na\textsuperscript{+} current in heart cells is often impractical, because the cardiac Na\textsuperscript{+} channel is incompletely blocked for TTX concentrations as high as 300 \textmu mol/L (eg, Reference 6). We present 2 complementary strategies to overcome these obstacles. First, we characterized the 2 known cardiovascular T-type isoforms expressed heterologously in HEK 293 cells. HEK 293 cells used in our studies are devoid of endogenous I\textsubscript{Na}. Second, we identified a subpopulation of atrial myocytes from an atrial tumor line (AT-1 cells) that expresses no Na\textsuperscript{+} current and little or no L-type Ca\textsuperscript{2+} current. The AT-1 cell is derived from murine right atrial myocytes that were immortalized by transformation with the simian virus 40 large T-antigen.\textsuperscript{7} Although most native cardiac myocyte preparations have overlapping Na\textsuperscript{+} current and L-type Ca\textsuperscript{2+} current, 2 exceptions, notably, nodal cells\textsuperscript{8} and embryonic ventricular myocytes,\textsuperscript{9} contain relatively high T-type channel density with respect to Na\textsuperscript{+} and L-type channels. Interestingly, both of these cell types are capable of autonomic activity. In the present study, we show that AT-1 cells have an unusually high relative density of T-type current. These cells also exhibit spontaneous action potentials.\textsuperscript{10} As in developing...
heart, the AT-1 Na⁺ current increases progressively with time in culture.¹¹ AT-1 cell repolarization currents are similar to normal atrial myocytes.¹⁰ The ultrastructure of AT-1 cells is also similar to normal atrial myocytes, including the presence of myofilbrils and large secretory granules.¹²,¹³

The ability of LVA, or T-type Ca²⁺, channels to modulate neuronal upstroke⁴⁻¹⁷ and to promote cardiac pacemaking⁸,¹⁹ underscores the importance of understanding the similarities and distinctions of the 2 principal cardiovascular T-type Ca²⁺ channel isoforms. In this report, we show that functional characteristics of the α1G clone are similar to that expressed in atrial myocytes. Furthermore, we identify signature electrophysiological properties of α1G and α1H. This is important, because determining isoform-specific channel properties will allow us to predict functional roles from immunocytochemical studies on intact tissue that are not particularly accessible to patch-clamp recordings. The present study shows that the α1G channel is distinguished by a 10-fold faster recovery from inactivation than the closely related α1H channel. AT-1 cells also have fast recovery from inactivation and express the α1G channel. Because recovery from inactivation kinetics contributes to the refractory period, the isoform-specific recovery from inactivation has important implications for LVA channel–induced bursting in neurons and for pacemaking in cardiac myocytes.

Materials and Methods

Cell Culture

HEK 293 Cells

The α1H cDNA in the vector pCDNA3 was used to establish a stably transfected HEK 293 cell line. Cells are maintained in DMEM and 10% FBS, with 100 U/mL penicillin and 100 µg/mL streptomycin (GIBCO-BRL), as well as 1.0 mg/mL G-418 (GIBCO-BRL). α1G stable transformants were the same as those used in Lee et al.⁴

AT-1 Cells

AT-1 cells were generously provided by Dan Roden (Vanderbilt University, Nashville, Tenn) with permission from Loren Field (Indiana University, Indianapolis, Ind). Single AT-1 cells were propagated and obtained from tumors as described in detail by Yang et al.¹⁰ Only isolated cells from primary cultures were selected for study. In some cases, cells were trypsinized and replated a day before recordings. Cells were used 4 to 6 days after initial culturing. As with HEK recordings, medium was replaced with several rinses of recording bath solution immediately before patch-clamping. We observed a variety of electrical phenotypes of cultured AT-1 cells. For this study, we selected cells with no Na⁺ current and with negligible L-type Ca²⁺ currents (see Results). Potassium currents were not observable, because we used internal cesium and external tetraethylammonium (TEA) to block K conductances.

Electrophysiology

Solutions

Immediately before experiments, culture medium was replaced with the whole-cell bath recording solution consisting of (in mmol/L) NaCl 140, CsCl 15, KCl 2.5, TEA-Cl 10, CaCl₂ 2.5, MgCl₂ 1, HEPES 5, and glucose 5 (pH 7.4). The pipette contained (in mmol/L) K-glucosionate 110, CsCl 40, MgCl₂, 1, EGTA 3, and HEPES 5 (pH 7.35 with CsOH). Experiments were performed in the presence of 30 µmol/L TTX at room temperature (20°C to 22°C). Electrode resistance ranged from 1.5 to 2 MΩ. Currents were recorded with the whole-cell configuration of the patch-clamp technique. Recordings were initiated 5 minutes after patch break to allow equilibration of the patch pipette solution with the intracellular milieu. Analog series resistance compensation to 80% to 90% was used. Currents were filtered at 10 kHz (~3 dB) and sampled at 50 kHz. The pClamp6.04 and pClamp8.02b suite of programs was used for data acquisition and analysis.

Data Analysis

Voltage protocols are described in the Results section. Curve fitting was performed with the Origin 4.1 program (MicroCal). For current-voltage (I-V) curves, we used a Boltzmann distribution of the form

\[ I(V) = \frac{G_{\text{max}} \cdot (V - E_{\text{rev}})}{1 + \exp(V_{0.5} - V/k)} \]

where \( G_{\text{max}} \) is the maximal conductance, \( E_{\text{rev}} \) is the reversal potential, \( V_{0.5} \) is the midpoint, and \( k \) is the slope factor. For I-V curve fitting, only points >20 mV from reversal potential were considered in the fitting because of permutation effects at weak driving force.²⁰

Statistical Analysis

Fitted parameters are presented as mean±SEM. Initially, ANOVA was used to compare α1G, α1H, and AT-1. For significant differences (P<0.05), we applied a post hoc comparison using the Tukey honest significant difference for unequal N test (Statistica), and we present multivariate ANOVA P-values between groups.

Molecular Characterization

We used reverse transcription–polymerase chain reaction (RT-PCR) to determine which T-type channels are expressed in AT-1 cells. Total AT-1–cell RNA was prepared using Trizol (GIBCO-BRL). cDNA was prepared in a reaction containing 1 µg RNA, 10 mmol/L DTT, 2.5 µmol/L random hexanucleotide primers, 1 mmol/L each dNTP, 30 to 40 U RNAGuard (Pharmacia), and 40 U Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL). The first-strand cDNA was then used in a PCR reaction containing 100 pmol each primer and 2.5 U Taq polymerase (Pharmacia). Degenerate primers were designed to amplify the domain III–IV linker of all 3 T-type Ca²⁺ channels (upstream, GVVVEN, 5'-GGCCGTGCGTGCCTGGAAGACTT-3'; downstream, PINPTI, 5'-GATGATGGGGGGATTGAT-3'). PCR cycles consisted of 30 seconds at 94°C, 30 seconds at 58°C, and 1 minute at 72°C, repeated 30 times. PCR product was resolved on a 0.8% agarose gel, excised from the gel, and eluted using Qiaquick gel elution columns (Qiagen). The gel slice was melted in elution buffer and then passed over a DNA binding resin in a spin column. The resin was rinsed, and then the bound DNA was eluted in a small volume of low-salt buffer. The purified DNA was then cloned into pCR2.1 using the TA Cloning Kit (Invitrogen). Multiple individual colonies were expanded, and plasmid sequencing was done using Sequenase version 2.0 (Amersham).

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results

Voltage Dependence

The design of this study was to first compare the cardiovascular system T-type Ca²⁺ channels α1G and α1H in identical backgrounds. Second, with this information we addressed whether atrial myocyte \( I_T \) expresses signature α1G or α1H properties. The low voltage range of activation is an important defining feature of T-type Ca²⁺ channel currents. Under identical recording conditions, the 2 cardiovascular T-type channels, α1G and α1H, begin to noticeably activate at the same potential. We used 2 protocols to evaluate channel activation. The current-voltage curve in Figure 1 shows current beginning to activate at \( \approx 60 \) mV for both isoforms. In contrast to L-type Ca²⁺ channel current, the superimposed current traces cross over with increasing depolarization (Figures 1A through 1B). The peak I-V curve is fit by a modified Boltzmann distribution (see Materials and Methods) with a midpoint of \(-44±2\) mV for α1G (n=8) and \(-44±1\) mV for
Similar results are observed from activation curves drawn from tail current analysis (Figure 2). Both \( \alpha \)1G and \( \alpha \)1H activate at -60 mV. The midpoint (\( V_{0.5} \)) and slope (\( k \)) of the Boltzmann distributions for the instantaneous tail current activation curve are the same for these 2 isoforms.

To explore whether the voltage dependence of the cloned \( \alpha \)1G and \( \alpha \)1H channels reproduces \( I_T \) in a more native environment, we measured steady-state activation and inactivation from AT-1 cells in culture. Depolarizing steps from a \( V_{\text{hold}} \) of -100 mV elicit currents with voltage dependence strikingly similar to that of \( \alpha \)1G and \( \alpha \)1H in HEK 293 cells (Figures 1A through 1C and 2A through 2C). The current begins to noticeably activate at -65 to -60 mV and becomes progressively faster with depolarization (Figure 1C). The midpoints of activation and inactivation for \( I_T \) in AT-1 cells are -48 ± 2 mV (n=4) and -64 ± 2 mV (n=7), respectively. There is no significant difference in the voltage dependencies for either activation or inactivation between heterologously expressed \( I_T \) current and AT-1 cell currents (Figure 1G).

A major experimental problem for studying T-type currents in native preparations is the overlap of contaminating currents, including Na\(^+\) current and L-type Ca\(^{2+}\) current. We selected AT-1 cells that did not have Na\(^+\) current. Most AT-1 cells with large T-type current also displayed negligible L-type current (Figure 1F). To assess L-type current we prepulsed the cell to -40 mV to inactivate \( I_T \) (eg, see Figure 3). Some AT-1 cells showed a small L-type current that did not activate until -5 mV (Figure 1F). Unlike primary isolated myocytes, AT-1 cells under our recording conditions rarely exhibited a larger L-type current. In addition, the activation range of L-type current in AT-1 cells is clearly distinct from \( I_T \).

Prolonged depolarization elicits steady-state inactivation of \( \alpha \)1G and \( \alpha \)1H. In a separate series of experiments, we measured the midpoint of inactivation with increasing duration of a prepulse potential. For durations <3 seconds, there is a depolarizing shift of the inactivation curve. Therefore, to measure current at steady state we used a 5-second prepulse.

**Figure 1.** \( \alpha \)1G, \( \alpha \)1H, and AT-1 \( I_T \) have a similar macroscopic current-voltage dependence. A through C, Superimposed current traces from \( V_{\text{hold}} \) = -100 mV to \( V_{\text{test}} \) = -65 to +15 mV in 5-mV increments. Notice Na\(^+\) current-like crossover kinetics. Scale bars=20 ms and 250, 300, and 100 pA for \( \alpha \)1G, \( \alpha \)1H, and AT-1 \( I_T \), respectively. D and E, Peak I-V curves fitted to a Boltzmann distribution (smooth curve) for \( \alpha \)1G and \( \alpha \)1H. F, AT-1 \( I_T \) peak I-V curve for \( V_{\text{hold}} \) = -100 mV superimposed on \( V_{\text{hold}} \) = -40 mV. Notice relatively small amplitude of Ca\(^{2+}\) current activating at -5 mV. G, Summary of fit parameters for curves in panels D through F. There are no significant differences in midpoint (\( V_{0.5} \)), slope factor (\( k \)), or fitted reversal potential (\( E_{\text{rev}} \)). n=8, 6, and 4 for \( \alpha \)1G, \( \alpha \)1H, and AT-1 \( I_T \), respectively.

**Figure 2.** \( \alpha \)1G, \( \alpha \)1H, and AT-1 \( I_T \) have similar activation curve voltage dependence. Shown are activation curves drawn from tail currents. A through C, Current traces elicited by a 10-ms prestep ranging from -75 to +20 mV in 5-mV increments followed by a return step to -80 mV. Current at -80 mV is well described by a single exponential function. D through F, Instantaneous amplitude of tail current at -80 mV plotted vs voltage is fitted with a Boltzmann distribution. G, Boltzmann fit parameters show no significant difference among \( \alpha \)1G, \( \alpha \)1H, and AT-1 \( I_T \). n=11, 9, and 6 for \( \alpha \)1G, \( \alpha \)1H, and AT-1, respectively.
The voltage dependence of steady-state inactivation is not significantly different between α1G and α1H (Figures 3A through 3B). Inactivation is fitted to a Boltzmann distribution with $V_\text{h}$ of $-66 \pm 1$ mV (n = 21) and $-63 \pm 2$ mV (n = 16) for α1G and α1H, respectively (Figure 3).

### Macroscopic Inactivation and Deactivation Kinetics

In contrast to L-type Ca$^{2+}$ current, another major defining characteristic of T-type current is that for increasing depolarization the current decay crosses over (Figures 1A through 1C). This kinetic feature of $I_T$ is Na$^+$ channel-like, albeit ~10 times slower for macroscopic inactivation. Macroscopic inactivation is well described by a single exponential component for all potentials. The time constant of macroscopic inactivation decreases with depolarization and tends to be faster for α1G than for α1H (Figure 4). For potentials positive to $-30$ mV, the time constant is no longer voltage dependent. AT-1 $I_T$ is also well fitted by a single exponential for potentials up to $-5$ mV. For AT-1 cells only, for potentials $\geq 0$ mV, a second time constant of $5 \pm 1$ mV for $10$ ms, followed by a test potential ranging from $-60$ to $-160$ mV for α1G. The decay of tail current at these hyperpolarized potentials represents deactivating T-type current and is well fit by a single exponential function with voltage-independent offset that is slightly faster for α1G, than AT-1 $I_T$, and than α1H, D. Pooled data of voltage-independent offset and slope factor for $\tau_{\text{activation}} (V)$. n = 8, 4, and 4 for α1G, α1H, and AT-1 $I_T$, respectively.

T-type Ca$^{2+}$ channel currents deactivate slowly in contrast to L-type channels. Figure 5A shows tail currents recorded after a 10-ms depolarization to 0 mV followed by a return to potentials ranging from $-60$ to $-160$ mV for α1G. The decay of tail current at these hyperpolarized potentials represents deactivating T-type current and is well fit by a single exponential function with voltage-independent offset and slope factor for $\tau$ (V). Offset suggests voltage-independent rate. E. Pooled summary for $\tau$ (V) voltage-independent offset and voltage dependence (slope factor). n = 6, 5, and 5 for α1G, α1H, and AT-1 $I_T$, respectively.

**Figure 3.** α1G, α1H, and AT-1 $I_T$ have the same steady-state inactivation voltage dependencies. To achieve steady-state, a 5-second prepulse ranging from −120 to −20 by 5 mV increments was followed by a test pulse to 0 mV. A through C, a 5-second prepulse ranging from −210 to −20 mV was followed by a test pulse to 0 mV. A through C, α1G (A), α1H (B), and AT-1 $I_T$ (C) inactivation curves, normalized to maximal current and fitted to a Boltzmann distribution (smooth curve). D, Fitted parameters of Boltzmann distribution pooled from cells show no significant difference for $V_\text{h}$ or slope factor. $n = 21, 16, 7$ for α1G, α1H, and AT-1 $I_T$, respectively.

**Figure 4.** Macroscopic inactivation kinetics and voltage dependence for α1G (A), α1H (B), and AT-1 $I_T$ (C). Single exponential fit parameters for the decaying phase of currents as shown in Figures 1A through 1C. Voltage dependence of the macroscopic inactivation rate is well described by a single exponential function with voltage-independent offset that is slightly faster for α1G, than AT-1 $I_T$, and than α1H. D, Pooled data of voltage-independent offset and slope factor for $\tau_{\text{activation}} (V)$. n = 8, 4, and 4 for α1G, α1H, and AT-1 $I_T$, respectively.

**Figure 5.** Deactivation of T-type Ca$^{2+}$ current determined from tail relaxation rate. A, Current in α1G elicited by a prepulse to 0 mV for 10 ms, followed by a test potential ranging from $-60$ to $-160$ mV in 10-mV increments. Current decay is well fitted by a single exponential function with $\tau$ vs voltage shown for α1G (B), α1H (C), and AT-1 $I_T$ (D). Smooth curves in panels B through D are single exponential fit with an offset for $\tau$ (V). Offset suggests voltage-independent rate. E, Pooled summary for $\tau$ (V) voltage-independent offset and voltage dependence (slope factor). n = 6, 5, and 5 for α1G, α1H, and AT-1 $I_T$, respectively.
exponential. Despite a negligible contaminating L-type current in some AT-1 cells, we adequately fit AT-1 tail currents to a single exponential. In turn, the $\tau_{\text{decay}}$ curve (Figures 5B through 5D) is fitted with a single exponential with an offset for $\alpha_1G$, $\alpha_1H$, and AT-1. As with native T-type channels, $\alpha_1G$ and $\alpha_1H$ show a voltage-independent $\tau_{\text{decay}}$ for large hyperpolarizations, suggesting a gating scheme with a voltage-independent transition from the open to the closed states. Although the voltage-independent deactivation time constant is $\approx$ 5 times slower for $\alpha_1H$ (1.04±0.40 ms, n=5) than for $\alpha_1G$ (0.20±0.03 ms; n=6), there is more variability in the $\alpha_1H$ data (Figure 5E). In comparison with the cloned T-type channels, the AT-1 current decays with the slower kinetics similar to those of $\alpha_1H$ (1.17±0.14 ms, n=5). The voltage dependence of deactivation is similar for the cloned channels but steeper for AT-1 $I_f$ (Figure 5E).

**Recovery From Inactivation Provides an Isoform-Specific Current Signature**

By analogy to Na$^+$ current, a key microscopic determinant for refractory period is the recovery from inactivation kinetics. This is a critical channel parameter to determine, particularly in cells that may use T-type Ca$^{2+}$ channels in lieu of, or in addition to, Na$^+$ current for depolarization. To measure recovery from inactivation, we prepulsed cells expressing $\alpha_1G$, $\alpha_1H$, or AT-1 $I_f$ for 1 second to 0 mV. We then returned to various recovery potentials ($V_{\text{recovery}}$) for a variable interval and measured peak current as a function of recovery interval (Figure 6A). After $\approx$ 3 seconds at a $V_{\text{recovery}}$ of −100 mV, recovery from inactivation is nearly complete and indistinguishable for all 3 preparations; however, there is a significantly faster recovery from inactivation for the $\alpha_1G$ compared with the $\alpha_1H$ for shorter recovery intervals (Figure 6A). AT-1 $I_f$ is nearly indistinguishable from $\alpha_1G$ recovery kinetics and is distinct from $\alpha_1H$. The $\alpha_1G$ and AT-1 recovery from inactivation curves could not be fitted by a single exponential function for recovery at −100 mV. Biexponential fits of recovery from inactivation yield time constants in the range of $\approx$ 100 ms and 1 to 2 seconds for each preparation (Figures 6B and 6C). There is no significant difference among the fast (Figure 6B) or slow time constants (Figure 6C) for the 3 preparations at $V_{\text{recovery}}$ of −100 mV. There is, however, a striking difference in the relative amplitude of the slow component of recovery. For $\alpha_1H$, 84±2% (n=10) of the total amplitude recovered with the slow (seconds) time constant; in contrast, only 24±3% (n=10) and 27±2% (n=7) of $\alpha_1G$ and AT-1 $I_f$, respectively, recovered with the slow (seconds) time constant (Figure 6D). The majority of $\alpha_1G$ and AT-1 $I_f$ recovered with time constants of 84±2 and 152±12 ms at $V_{\text{recovery}}$ of −100 mV.

In Na$^+$ channels, recovery from inactivation is voltage dependent, becoming faster with increasing hyperpolarization. In contrast, native $I_f$ studies suggested that T-type channel recovery from inactivation is weakly, if at all, voltage dependent. We tested recovery from inactivation for additional recovery potentials of −80 and −120 mV (Figures 6B through 6D). For recovery potentials from −100 to −120 mV there is weak, if any, voltage dependence to recovery from inactivation. For recovery at −80 mV, the fast time constant was slower than at more hyperpolarized potentials (Figure 6B), but the slow time constants were not significantly different from those at −100 or −120 mV (Figure 6C). Interestingly, there was a tendency for the fractional slow amplitude to increase with hyperpolarization for any given preparation. For $\alpha_1G$ at −80 mV, the slow amplitude reduced to nil, resulting in a single exponential fit. In summary, we conclude that the relative amplitudes of fast and slow components of recovery from inactivation, at hyperpolarized potentials, serve as a clear signature of T-type channel isoform expression. Because we do not yet know the structural determinants of this kinetic property, we cannot rule out the possibility that splicing variation contributes to the observed difference. Therefore, more detailed structure-function studies are warranted.
are able to amplify all 3 known T-type channels, 26 no other cells.25 Figure 7B shows the deduced amino acid sequence for primers on a range of tissues, this splice variant was found experiments in which RT-PCR was done with the same logical ionic conditions to deduce the functional role for T-type Ca\textsuperscript{2+} channel genes, 2–4 In studies (upper line) with the AT-1 PCR product (lower line). Primer sequences are underlined, transmembrane segments are marked, and alternative exons in the domain II–III loop are in boldface.

**Molecular Identification of T-Type Isoform in AT-1 cells**

To classify T-type channel isoform expression, we performed RT-PCR from cultures of AT-1 cells. The 3 T-type Ca\textsuperscript{2+} channel genes, \(\alpha\text{I}G\), \(\alpha\text{I}H\), and \(\alpha\text{II}\), have distinguishable amino acid sequences in the III–IV linker region.2–4 In addition, splice variations have been identified previously for each of these genes, discovered either by direct cloning or by PCR.4,24,25 Lee et al reported 4 variants of the \(\alpha\text{I}G\) III–IV linker, which exist as alternative exons in the genomic sequence for \(\alpha\text{I}G\). PCR of the III–IV interdomain linker from AT-1 cell RNA resulted in a single, prominent band (Figure 7A). The PCR product was excised and subcloned, and multiple isolates were sequenced to confirm their identity. Eight of 10 clones sequenced revealed that a splice variant of \(\alpha\text{I}G\) is predominant in AT-1 cells. Even though the primers negative control reaction done in the absence of reverse transcriptase to rule out contaminating genomic DNA. B, Alignment of deduced amino acid sequences of the rat \(\alpha\text{I}G\) splice variant (lower line). The rat brain sequence we expressed in HEK cells (upper line) is aligned with the \(\alpha\text{I}G\) III–IV linker, which exist as alternative exons in the genomic sequence (Figure 7A). Primer sequences are underlined, transmembrane segments are marked, and alternative exons in the domain II–III loop are in boldface.

**Discussion**

T-type Ca\textsuperscript{2+} current recordings must be made under physiological ionic conditions to deduce the functional role for \(I_T\). In most native preparations, this is impossible because of the expression of overlapping Na\textsuperscript{+} and, to a lesser degree, L-type Ca\textsuperscript{2+} currents. The inclusion of TTX (or saxitoxin) does not completely ameliorate cardiac Na\textsuperscript{+} current overlap, because with a half-block concentration of \(\sim 1 \mu\text{mol/L}\) (see, eg, Reference 6) and a large excess of Na\textsuperscript{+} current density, it is very difficult to completely eliminate cardiac myocyte Na\textsuperscript{+} current. One strategy to measure T-type current is to heterologously express the T-type channels on a null background. However, heterologous expression carries the unknown that a noncardiac myocyte background may modify channel properties. Therefore, it was important to compare cloned, heterologously expressed channel current with cardiac myocyte current. In this study, we showed that transfected atrial myocytes (AT-1 cells) express T-type channel current (\(I_T\)) with properties similar to the cloned T-type channels \(\alpha\text{I}G\) and \(\alpha\text{I}H\). Both of these channel clones are present in the cardiovascular system; therefore, either of these clones may be expressed by myocytes. We show that recovery from inactivation kinetics provide a clear discrimination of T-type channel isoform-specific expression. Although there is correspondence of molecular and electrophysiological properties between the \(\alpha\text{I}G\) isoform current and \(I_T\) in AT-1 cells, functional differences exist.

The distinct recovery from inactivation kinetics of \(\alpha\text{I}G\) from \(\alpha\text{I}H\) is a useful feature for identifying the T-type channel isoform expressed in AT-1 cells. Distinct recovery kinetics were previously noted in a variety of native preparations. In general, native \(I_T\) recovery kinetics can be separated into fast and slow recovery groups. For example, dorsal root ganglion cell27 and hypothalamic neuronal \(I_T\) recover with time constants measured in seconds; in contrast, cardiac SA nodal cell,29 atrial myocyte,30 sensory neuronal \(I_T\), and thalamocortical neuronal \(I_T\) recover with time constants ranging from 100 to 250 ms. It is tempting to assign \(\alpha\text{I}G\) and \(\alpha\text{I}H\) to each of these preparations. Indeed, recent localization studies have shown that \(\alpha\text{I}H\) is in nodusus ganglion cells,32 and \(\alpha\text{I}G\) is expressed in thalamic relay neurons.33

Recovery from inactivation includes the sum of the transitions from inactivated to closed channel states. In earlier native studies, recovery kinetics were fitted to a single exponential function.22 We, however, needed to use biexponential fits to describe the data. Surprisingly, \(\alpha\text{I}G\), \(\alpha\text{I}H\), and AT-1 \(I_T\) all had similar rates of recovery. This leads to an unexpected conclusion with respect to the molecular mechanisms underlying recovery from inactivation. For potentials negative to \(-100\text{ mV}\), there was no significant voltage dependence of recovery rates (\(-140\text{ and }-160\text{ mV};\) data not shown). For the \(-80\text{-mV} recovering potential, the fast rate of recovery was slower than at \(-100\text{ mV}\), and the onset to recovery was sigmoidal. This suggests a voltage-dependent step between inactivated states (compare Reference 33). Kuo and Bean34 have interpreted a delay in recovery from inactivation in Na\textsuperscript{+} channels as deactivation that must precede recovery from inactivation in Na\textsuperscript{+} channels. Multiple rates of recovery after the delay further suggest that a gating scheme has to include at least 2 voltage-independent transitions from open to closed states. The most recent T-type channel kinetic simulations incorporate these general principals but are inadequate, because they forecast that 95% of channels would be in the closed rather than the inactivated state at hyperpolarized potentials.35 This is at odds with single-channel recordings showing that the null fraction is on the order of 75%, implying that the only \(\sim 25\%\) of the channels are in the closed...
state at a hyperpolarized $V_{\text{m}}$ (J. Satin, unpublished data, 1999; compare References 21 and 22). The surprising point is that I-to-C transitions may be similar among T-type Ca$^{2+}$ channels. The T-type isoform–specific recovery from inactivation probably arises from differences in voltage-dependent transitions among inactivated states.

Our RNA-PCR data indicated that the primary T-type Ca$^{2+}$ channel expressed in AT-1 cells is a splice variant of $\alpha$1G. Although consistent with the kinetic data, we cannot rule out the possibility that other genes or splice variants are also present but were not detected in these experiments, even though the PCR primers have been demonstrated to amplify all known T-type channel genes from a variety of tissues and species (see Reference 24).

AT-1 cells are of interest both as a possible surrogate heart cell and as a model system for understanding the contribution of various ionic currents to the action potential. The ability of AT-1 cells to integrate in syngeneic transplantation and the spontaneous action potentials of single AT-1 cells argues for the continued study of these cells. We have noted, however, that there is a variable expression pattern of $I_{\text{Na}}, I_{\text{f}},$ and $I_{\text{Ca-L}},$ in AT-1 cells. In general, we have also noted increasing Na$^{+}$ current density with time in culture. In summary, these aspects of AT-1 cells in culture, a relatively large but diminishing $I_{\text{f}}$-to-$I_{\text{Ca-L}}$ ratio, an increase of $I_{\text{Na}}$, an increase of second messenger responsiveness, and a decrease of spontaneous activity, mimic the developmental sequence of cardiac myocyte excitability. The variable expression levels of various conductances, coupled with spontaneous cardiac-like action potentials, positions AT-1 cells as an excellent model system for testing the role of relative current densities in shaping the action potential.

References

Identification of a T-Type Ca\textsuperscript{2+} Channel Isoform in Murine Atrial Myocytes (AT-1 Cells)
Jonathan Satin and Leanne L. Cribbs

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