T-type calcium currents in rat cardiomyocytes during postnatal development: contribution to hormone secretion

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ABSTRACT

Among the several types of voltage-dependent Ca2+ channels identified in various cells, only L-type and T-type channels are expressed in cardiomyocytes. Although L-type channels have been shown to play a central role in the excitation-contraction coupling of cardiac myocytes, the function of T-type channels in the heart remains poorly understood. T-type Ca2+ currents (I_{Ca,T}) are observed in pacemaker cells, in which they are shown to participate in the electrogensis of impulse generation (5, 27), and are clearly described in embryonic or neonatal cultured atrial and ventricular myocytes, whereas they are rarely observed in adult ventricular myocytes, except in the guinea pig (15, 12), suggesting that T-type channels are associated with development and postnatal growth of cardiac cells.

Recently, full-length cDNAs encoding three homologous but distinct new α1-subunits (α1G, α1H, α1I) have been identified (4, 9, 17). Their expression in oocytes and mammalian cells gives rise to currents with all the typical properties of native T-type channels. Northern blot analyses have indicated that messengers for both α1G- and α1H-subunits were expressed in adult human heart (17, 4), whereas no I_{Ca,T} has been observed in human cardiac cells isolated from either atrial appendage or ventricular tissue (16). It is important to investigate whether cardiac I_{Ca,T} is related to these α1-isoforms.

In the present paper, we describe the postnatal evolution of T-type versus L-type Ca2+ currents in neonatal rat atrial and ventricular myocytes. Information on the molecular nature of the cardiac T-type channels is provided by comparison of the functional properties of neonatal cardiac I_{Ca,T} with those reported for recombinant α1G-, α1G-, α1H-, and α1I-subtype Ca2+ currents and by RT-PCR experiments. The results suggest a linkage between neonatal cardiac I_{Ca,T} and the α1G gene. The contribution of T-type currents to Ca2+ signaling in neonatal atrial cells was investigated by measuring the secretion of atrial natriuretic factor (ANF).

METHODS

Cell isolation. Single rat atrial and ventricular myocytes were enzymatically isolated following standard procedures. Four-day-old to adult rats of either sex were anesthetized with pentobarbital sodium. The heart was quickly removed and rinsed in a warm (37°C) Tyrode solution (mM: 112 NaCl, 6 KCl, 2 MgCl2, 4 NaHCO3, 1.5 KH2PO4, 5.85 glucose, and 5.85 pyruvic acid, and 5.85 glucose with 17.7 mg/l phenol red, 60 mg/l penicillin G, and 100 mg/l streptomycin, pH 7.5 adjusted with NaOH) supplemented with 2 mM Ca2+. The heart was then perfused retrogradely by aortic cannulation on a Langendorff system with a Ca2+-free Tyrode solution for 8 min.
followed by a 0.7 mg/ml collagenase (type II, Worthington) solution made with the Tyrode solution and containing 10 µM Ca²⁺. Perfusion solutions were warmed to 37°C, and the duration of the enzyme solution perfusion was varied with the age of the animal from which the heart had been removed, from 15 min for the youngest animals to 30 min for the oldest. At the end of the perfusion, the atria were separated from the ventricles and mechanical dissociation of the myocytes was performed with a smooth-tip Pasteur pipette. Cells were then maintained up to 8 h in a high-K⁺ medium containing (mM) 100 potassium glutamate, 10 potassium aspartate, 25 KCl, 10 K2HPO4, 2 MgSO4, 20 taurine, 5 creatine, 0.5 EGTA, 20 glucose, and 5 HEPES with 0.1% BSA (pH 7.2 adjusted with KOH) at 4°C.

Electrophysiological recordings. All experiments were conducted at room temperature (20–22°C). Whole cell Ca²⁺ currents were recorded from single myocytes using the patch-clamp method with a Biologic RK 300 or Axopatch 200A amplifier interfaced to a PC computer. Fire-polished pipettes were made from borosilicate glass and had a resistance of 2.5–3.5 MΩ. They were filled with an internal solution containing (mM) 130 CsCl, 10 EGTA, 25 HEPES, 3 ATP (Mg), and 0.4 GTP (Na) (pH at 7.2 with CsOH). Current recordings were performed in a bath solution containing (mM) 2 CaCl₂, 5 4-aminopyridine, 136 tetraethylammonium (TEA)-Cl, 1.1 MgCl₂, 25 HEPES, and 22 glucose with 17.7 mg/l phenol red (pH 7.4 with TEA-OH). Data were filtered at 5 kHz. Mibefradil (Produits Roche) was prepared freshly and directly dissolved in the external recording solution. NiCl₂ was directly dissolved in the external solution. NiCl₂ was placed onto filters (0.45-m Acrodisc LCPVDF, Gelman Sciences) and perfused (Minipulse peristaltic pump, Gilson) for 45 min with normal Locke buffer at a flow rate of 50 µl/min. The flow rate was slowly increased during this period to 100 µl/min. Collection of the perfusate over 5-min periods started 60 min after the tissues were loaded onto the filter. The whole set-up was mounted in an incubator, and the perfusion experiments were performed at 37°C. ANF content in each fraction was then determined by a competitive RIA using a RIA kit purchased from Phoenix Pharmaceuticals (RK-005–24). Depolarization medium contained 50 or 20 mM K⁺ (final concentration), and a constant Na⁺ concentration was maintained by equimolar substitution of KCl for N-methyl-D-glucamine chloride. The results presented correspond to the average ANF content of standard aliquots taken from the collected fractions arising from at least three separate groups of tissues. The amount of hormone released during each period was calculated by subtracting the amount of hormone released under basal conditions from that observed during and directly after the stimulus. Student’s t-test was used for statistical tests.

mRNA preparation and RT-PCR analysis. Total RNA preparations from cerebellum, atrial, and ventricular tissues of 8-day-old animals and from kidney, skeletal muscle, cerebellum, atrial, and ventricular tissues of adult rats were done using TRIzol (Life Technologies) according to the manufacturer’s protocol. The primers used are noted in Table 1.

RESULTS

Ca²⁺ currents in neonatal cardiac myocytes. Figure 1A illustrates representative Ca²⁺ currents recorded from freshly dissociated 8-day-old rat atrial myocytes in response to increasing depolarizations. At a HP of -100 mV, IₚCa,T is activated by a depolarization around -50 mV and peak current is observed around -30 mV.
For test potentials above $-30$ mV, an L-type current is activated that displays a maximum amplitude near $110$ mV. At a HP of $-50$ mV, $I_{\text{Ca,T}}$ is mostly inactivated whereas the L-type current remains almost unaffected. The difference between current traces at HP of $-50$ mV and $-100$ mV for each potential reflects the $I_{\text{Ca,T}}$. Figure 1B represents the mixed current-voltage curve of T-type and L-type currents present at a HP of $-100$ mV as well as the current-voltage curve of the L-type current recorded at a HP of $-50$ mV; the difference between the two curves indicates the theoretical $I_{\text{Ca,T}}$-voltage relationship. Both T-type and L-type currents were observed in atrial and ventricular cells at this age. $I_{\text{Ca,T}}$ was always present with a larger amplitude than L-type current in atrial myocytes. In contrast, $I_{\text{Ca,T}}$ was not systematically observed in ventricular cells, and its amplitude was always smaller than that of L-type current. Application of the $\beta$-adrenergic agonist isoproterenol (2 $\mu$M) during a double test pulse protocol activating either T-type current or mostly L-type current had no effect on the T-type current, whereas a large current increase (from 70 to 160 pA)

### Table 1. Specific primers used in PCR experiments

<table>
<thead>
<tr>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Annealing Temperature, °C</th>
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<tr>
<td>$a_{1T}$</td>
<td>5'-atgttgatgtgcatgtgaac-3'</td>
<td>5'-geacgctgtgatgtgcagcag-3'</td>
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<tr>
<td>$a_{1G}$</td>
<td>5'-gtcttctgctgcatgtgaac-3'</td>
<td>5'-gcacgcggttgatgagctttgag-3'</td>
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<tr>
<td>$a_{1H}$</td>
<td>5'-gagttcggtgacagtgtgagc-3'</td>
<td>5'-cattccagagggcagacagttag-3'</td>
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<tr>
<td>$a_{1C}$</td>
<td>5'-cattccagagggcagacagttag-3'</td>
<td>5'-agaagatgtgatgtgtgtgaag-3'</td>
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</table>

For test potentials above $-30$ mV, an L-type current is activated that displays a maximum amplitude near $+10$ mV. At a HP of $-50$ mV, $I_{\text{Ca,T}}$ is mostly inactivated whereas the L-type current remains almost unaffected. The difference between current traces at HP of $-50$ mV and $-100$ mV for each potential reflects the $I_{\text{Ca,T}}$. Figure 1B represents the mixed current-voltage curve of T-type and L-type currents present at a HP of $-100$ mV as well as the current-voltage curve of the L-type current recorded at a HP of $-50$ mV; the difference between the two curves indicates the theoretical $I_{\text{Ca,T}}$-voltage relationship. Both T-type and L-type currents were observed in atrial and ventricular cells at this age. $I_{\text{Ca,T}}$ was always present with a larger amplitude than L-type current in atrial myocytes. In contrast, $I_{\text{Ca,T}}$ was not systematically observed in ventricular cells, and its amplitude was always smaller than that of L-type current. Application of the $\beta$-adrenergic agonist isoproterenol (2 $\mu$M) during a double test pulse protocol activating either T-type current or mostly L-type current had no effect on the T-type current, whereas a large current increase (from 70 to 160 pA)
Fig. 2. Evolution of Ca\(^{2+}\) current density. A: current-voltage relationship recorded from HP –100 mV on freshly isolated atrial myocytes at 8 days (n = 51) and 3 wk (n = 11) and in adult (4 mo; n = 11) and on freshly isolated ventricular myocytes at 8 days (n = 9) and 3 wk (n = 6) and in adult (2 mo; n = 6). B: histograms of peak current density amplitudes for freshly isolated atrial myocytes at 4 days (n = 14), 8 days (n = 51), and 3 wk (n = 11) and in adult of 4 mo (n = 11) and for ventricular myocytes at 8 days (n = 9) and 3 wk (n = 6) and in adult of 2 mo (n = 6). Maximum amplitudes were measured at –30 mV for the \(I_{Ca,T}\) (filled bars) and at +10 mV for the L-type current (open bars). Data are shown as means ± SE.
for the higher depolarization was related to enhancement of the L-type current amplitude (Fig. 1C). This result mainly indicates that there is no significant contribution of L-type current at a depolarization of −30 mV, where \( I_{\text{Ca,T}} \) is maximally activated.

Postnatal evolution of \( I_{\text{Ca,T}} \) density. T-type and L-type Ca\(^{2+}\) current density were measured at the peak current (−30 and +10 mV, respectively) in atrial and ventricular myocytes from 4-day-old to adult rat hearts (Fig. 2). \( I_{\text{Ca,T}} \) in atrial cells is already predominant in 4-day-old myocytes, with a density of 4.52 ± 0.63 pA/pF (\( n = 14 \)). However, \( I_{\text{Ca,T}} \) is maximal in 8-day-old rat atrial myocytes, with a density of 5.78 ± 0.38 pA/pF (\( n = 51 \)). The density of the \( I_{\text{Ca,T}} \) decreased by 2.44 ± 0.27 pA/pF after 3 wk (\( n = 11 \)) and was low in adult rat atrial myocytes (\( n = 11 \)). The situation is different in ventricular cells because \( I_{\text{Ca,T}} \) is also observed but has a smaller amplitude (vs. L-type) in 8-day-old rats (1.47 ± 0.26 pA/pF, \( n = 9 \)) and then disappears in ventricular cells from 3-wk-old rats. We did not detect any major changes for \( I_{\text{Ca,T}} \) activation and inactivation properties in the different stages investigated (see Table 2).

Investigation of molecular nature of cardiac \( I_{\text{Ca,T}} \). RT-PCR analysis was performed using reverse-transcribed products from mRNA collected from rat neonatal and adult cardiac tissues as well as from other tissues (skeletal muscle, cerebellum, kidney) used as positive or negative controls. Specific primers (see METHODS) were designed to detect the presence of \( \alpha_{1E} \), \( \alpha_{1G} \), \( \alpha_{1H} \), and \( \alpha_{1C} \) in heart and other selected tissues. Figure 3 shows that \( \alpha_{11} \) mRNA is not present in the heart but is found in the cerebellum as expected (9), whereas \( \alpha_{1G} \) and \( \alpha_{1H} \) mRNAs are both present in the atrial and ventricular tissues of young and adult rats. We also detected \( \alpha_{1E} \) mRNA in atrial tissue from young rats. As expected from previous studies, our control experiments demonstrated the presence of \( \alpha_{1C} \) in both cerebellum and cardiac tissues, the presence of \( \alpha_{11} \) in the kidney but not in the cerebellum, and the presence of \( \alpha_{1G} \) in the cerebellum but not in the kidney (4).

**Table 2. Activation and inactivation characteristics of cardiac calcium currents**

<table>
<thead>
<tr>
<th></th>
<th>Activation</th>
<th>Inactivation</th>
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<tr>
<td></td>
<td>( V_{50,T} )</td>
<td>( k_T )</td>
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<tr>
<td>Atrial myocytes</td>
<td></td>
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<tr>
<td>4 days</td>
<td>−42.14 ± 0.9</td>
<td>6.27 ± 0.9</td>
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<td>(n = 14, N = 2)</td>
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<tr>
<td>8 days</td>
<td>−49.98 ± 0.84</td>
<td>5.16 ± 0.4</td>
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<tr>
<td>(n = 51, N = 10)</td>
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<tr>
<td>3 wk</td>
<td>−43.56 ± 1.5</td>
<td>5.22 ± 0.87</td>
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<tr>
<td>(n = 11, N = 1)</td>
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<tr>
<td>Adult (4 mo)</td>
<td>−42.26 ± 9.65</td>
<td>6.92 ± 4</td>
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<tr>
<td>(n = 11, N = 1)</td>
<td></td>
<td></td>
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<tr>
<td>Ventricular myocytes</td>
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<tr>
<td>8 days</td>
<td>−43.98 ± 5.1</td>
<td>4.76 ± 3.2</td>
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<tr>
<td>(n = 9, N = 4)</td>
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<tr>
<td>3 week</td>
<td>−12.55 ± 0.53</td>
<td>5.72 ± 0.37</td>
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<tr>
<td>(n = 6, N = 1)</td>
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<tr>
<td>Adult (2 mo)</td>
<td>−16 ± 0.6</td>
<td>5.14 ± 0.43</td>
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<td>(n = 6, N = 2)</td>
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Values are means ± SE; \( n \), no. of cells; \( N \), no. of rats. T, T-type Ca\(^{2+}\) current; L, L-type Ca\(^{2+}\) current; \( V_{50,T} \), potential of half-maximal activation; \( k \), slope of voltage dependence of activation; ND, not determined.

Biophysical properties of cardiac \( I_{\text{Ca,T}} \). \( I_{\text{Ca,T}} \) properties were studied in atrial myocytes from 8-day-old rats at a depolarization of −30 mV, at which \( I_{\text{Ca,T}} \) is maximally activated without a significant contribution of L-type current. The current activates more rapidly with increasing depolarizations, the time to peak values ranging around 25 ms for a potential of −50 mV to <10 ms for a test potential above −50 mV (Fig. 4A). As previously reported in various cell types, \( I_{\text{Ca,T}} \) also inactivates faster with depolarization, with a time constant of 12.9 ± 0.4 ms at −30 mV. Measurements were not performed for higher depolarizations because of contamination by L-type current. The steady-state in...
activation relationship obtained using a classical double-pulse protocol indicates a half-inactivation potential of $-68.4 \text{ mV}$ with a slope of $-4.09 \text{ mV}$. The normalized values of steady-state activation (obtained from the current-voltage curve) and inactivation (Fig. 4B) reveal a window current between $-65$ and $-45 \text{ mV}$. Membrane repolarization at the time to peak current displays slow deactivating tail currents, a hallmark of T-type currents, because the time constant is $1.3 \text{ ms}$ for repolarization at $-100 \text{ mV}$ and $4.1 \text{ ms}$ at $-60 \text{ mV}$ (Fig. 4C).

**Pharmacology of cardiac $I_{\text{Ca,T}}$.** Several ions and molecules have been used to distinguish between L-type and T-type Ca$^{2+}$ channels such as Ni$^{2+}$ or mibefradil, a nondihydropyridine compound considered the most selective T-type versus L-type channel blocker (11). We found that the cardiac T-type channel is poorly sensitive to Ni$^{2+}$ because the apparent $K_d$ for inhibition [as shown by 50% inhibitory concentration (IC$_{50}$)] is $-160 \text{ M}$ (Fig. 5A). Moreover, Ni$^{2+}$ could not be used to separate the L-type from the T-type current because the IC$_{50}$ for L-type channel inhibition was a similar concentration (192 M; not shown). The inhibition of $I_{\text{Ca,T}}$ by mibefradil occurred with an IC$_{50}$ of 0.1 M (Fig. 5B). From experiments using an antisense strategy, it was previously suggested that the neonatal cardiac $I_{\text{Ca,T}}$ might be related to the $\alpha_{1E}$-subunit (18). We also tested the effect of SNX-482, a recently described potent specific blocker of recombinant $\alpha_{1E}$ currents (14). No effect on cardiac $I_{\text{Ca,T}}$ or expressed human $\alpha_{1G}$ T-type current was observed in the presence of 100 nM SNX-482, whereas the same concentration of toxin, as expected, strongly inhibited $\alpha_{1E}$-generated current in HEK-293 cells (Fig. 5C).

$I_{\text{Ca,T}}$ contribution to ANF secretion. To determine whether T-type channels might contribute to a physiological function such as hormonal secretion, ANF release was measured with peptide-specific RIA in perfused atrial tissue of 8-day-old rats. Figure 6A shows that membrane depolarization evoked by a high concentration of K$^+$ (50 mM) induced a large increase in ANF secretion from $4.1 \pm 0.2$ to $56.4 \pm 7 \text{ pg/15 min}$. The same protocol applied in the presence of 5 mM nitrendipine, which at this concentration blocks L-type without affecting T-type Ca$^{2+}$ current (not shown), leads to $21.78 \pm 2.5 \text{ pg/15 min}$ of ANF secretion, which corresponds to a large inhibition (61%). Application of 1 mM mibefradil, which preferentially blocks $I_{\text{Ca,T}}$ at this concentration, almost abolished ANF secretion (6.03 $\pm$ 0.8 pg/15 min, equivalent to 89% inhibition). Another set of experiments was performed using 20 mM K$^+$ to induce a weaker depolarization activating mostly T-type channels (19). A significant increase of ANF release was induced by application of the 20 mM K$^+$ solution from $4.9 \pm 0.4$ to $14.4 \pm 1.8 \text{ pg/15 min}$. This release is $25\%$ lower than the value obtained with 50 mM K$^+$ (Fig. 6B). In these experimental conditions, nitrendipine did not significantly block the evoked ANF release ($12.03 \pm 0.5 \text{ pg/15 min}$) whereas ANF release was completely abolished by the application of mibefradil (1 M). It is also interesting to note the existence of a basal level of ANF secretion (Fig. 6C). The application of 1 M mibefradil also reduced basal

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**Fig. 4.** Biophysical characteristics of $I_{\text{Ca,T}}$ from 8-day-old atrial myocytes. A: time to peak of Ca$^{2+}$ currents in atrial cells ($n = 12$). B: voltage-dependent activation and inactivation curves of $I_{\text{Ca,T}}$. The steady-state inactivation curve ($n = 32$, smooth curve) is fitted using a simple Boltzmann function [$V_{0.5} = -68.4 \text{ mV}$, slope factor ($k$) = $-4.09 \text{ mV}$], whereas activation curve is constructed from values of half-activation potential [$V_{0.5} = -41 \text{ mV}$] and with the slope factor ($k_r$ = 5.16 mV) obtained from the fit of the current-density voltage curve. C: typical recordings illustrating deactivation kinetics of $I_{\text{Ca,T}}$. Activation at $-30 \text{ mV}$ is followed by repolarization to $-100 \text{ mV}$ and $-60 \text{ mV}$. Time constants of deactivation are 1.3 and 4.1 ms, respectively; $n = 5$. 

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**Fig. 5.** IC$_{50}$ for inhibition of $I_{\text{Ca,T}}$ by mibefradil. Panel A: mibefradil concentration-response relationship obtained using a classical double-pulse protocol. Panel B: half-inactivation potential from the fit of the current-density voltage curve.
ANF release by 33.5% (from 4.9 ± 0.4 to 3.26 ± 0.1 pg/15 min).

**DISCUSSION**

We report here the postnatal evolution of \( I_{\text{Ca,T}} \) density in freshly dissociated atrial and ventricular myocytes from rat heart. This current exhibits the typical properties of T-type currents; in particular, slowly deactivating tail currents are observed during membrane repolarization. An overlap between the steady-state activation and inactivation relationships, i.e., a window current, indicates that a fraction of the T-type channel remains open and might generate a maintained \( \text{Ca}^{2+} \) influx in the voltage range between -65 and -45 mV. Our results show that \( I_{\text{Ca,T}} \) density in atrial cells peaks at postnatal day 8. It must be noted that in all cases, when present, \( I_{\text{Ca,T}} \) had similar biophysical properties. Our results differ from those of Xu and Best (26), who found a maximum \( I_{\text{Ca,T}} \) density in 5-wk-old rat atrial myocytes. They did not record \( \text{Ca}^{2+} \) currents before postnatal week 3, and the LVA \( \text{Ca}^{2+} \) current density reported in their experiments is four times lower than the density observed here 1 wk after birth.
Cardiac T-type channels in 8-day-old cardiomyocytes are blocked by a micromolar concentration of mibebradil (IC_{50} = 0.1 μM), as expected from previous studies on cardiovascular cells (11). Moreover, we found that neonatal I_{Ca,T} was poorly sensitive to Ni^{2+} (IC_{50} = 160 μM). Several genes coding for a T-type channel pore-forming subunit (α_{1G}, α_{1H}, α_{1I}) have been identified in rat, mouse, and human (4, 8, 9, 13, 17). Among these genes, α_{1H} mRNA was found in adult human heart (4) and α_{1G} was also detected in rat and human heart (17). However, α_{1I} was initially considered to be the cardiac T-type isoform. It is interesting to note that α_{1H} and α_{1G}-related currents in expression systems strongly differ in their Ni^{2+} sensitivity (IC_{50} = 13 and >150 μM, respectively; Refs. 10, 13), a property that may be considered as a signature to establish correlations between native and recombinant channels. Thus the Ni^{2+} sensitivity of the neonatal atrial I_{Ca,T} reported here is much closer to the value reported for α_{1G}-related currents. This result suggests that α_{1G} is related to the cardiac I_{Ca,T} expressed in rat neonatal cells. Several studies have reported that in adult myocytes of rabbit, frog, cat, and guinea pig, cardiac I_{Ca,T} are completely blocked by low Ni^{2+} concentration (40 μM) (2, 5, 12, 15, 27). We cannot exclude the possibility of different T-type isoform expression among various species. A developmental switch between isoforms is likely and is consistent with the recent report by Monteil et al. (13) demonstrating developmental regulation of α_{1G} transcript in human heart.

A previous study supported the idea that cardiac I_{Ca,T} may be related to the α_{1E} gene (18) although the experimental conditions differed in the sense that I_{Ca,T} expression was enhanced by hormone treatment. Our experiments do not support this hypothesis, because we show here the absence of an effect of SNX-482 on I_{Ca,T}. A recent study reported the existence of a SNX-482-resistant R-type current (23), but the permeation and conductance properties of the channel underlying this current are clearly different from any genuine T-type channel. RT-PCR analysis was performed on neonatal and adult rat atrial and ventricular tissues to determine the molecular make-up of T-type channels. Although the presence of α_{1E} mRNA is detected in 8-day-old cardiomyocytes, this may indicate that the protein is not targeted to the cell surface or is not functional. Moreover, atrial and ventricular tissues used for mRNA preparation could contain intracardiac neurons known to express several Ca^{2+} channels including the R-type channel assumed to be encoded by the α_{1E} subunit (6). As expected (9), the α_{1G} isoform is not expressed in cardiac tissue from either young or adult rats. Although I_{Ca,T} are not detected in adult ventricular myocytes, both α_{1G} and α_{1H} transcripts are present in neonatal and adult atrial and ventricular tissues. This might indicate that unidentified factors or unidentified subunits may control T-type gene expression or function during development. This is consistent with the report by Xu and Best (25) of the enhancement of the cardiac I_{Ca,T} by growth hormone. We cannot exclude that such a factor interferes with the targeting of the protein to the membrane or interacts with the T-type channel protein to inhibit its activity. Altogether, our data on the molecular and pharmacological characteristics of the neonatal cardiac T-type channel strongly suggest that it is encoded by the α_{1G} gene. This is in agreement with the recently published work of Satin and Cribbs (20), who reported the α_{1H} isoform as supporting the T-type current in a cell line derived from mouse atrial tissue.

The physiological role of the T-type channel in atrial cells remains unclear. It is often proposed to be involved in pacemaking activity in the heart, more specifically, in
sinoatrial node cells (5). In other cell types, T-type channels have been shown to contribute to Ca\(^{2+}\)-dependent hormone secretion such as aldosterone in adrenal glomerulosa cells (3, 19) or insulin in a pancreatic \(\beta\)-cell line (1). Cardiac tissue is known to be the main source of ANF release, a key regulator in the homeostasis of salt and water and in the maintenance of blood pressure, which in the normal adult heart is mainly restricted to both atria. Substantial changes in ANF gene expression take place at the time of birth, the ANF gene being highly expressed during fetal life both in atria and ventricle. A peak of ANF mRNA is observed in atrial tissue the first day after birth followed by a progressive decrease over 2 wk to reach near-adult levels (24). ANF secretion was shown to be stimulated by several factors, including atrial stretch and vasoactive agents such as angiotensin II, endothelin, or vasopressin, and most studies found that their action occurred via an elevation of the intracellular Ca\(^{2+}\) concentration (22). Our results show that ANF secretion is sensitive to dihydropyridine, confirming that L-type Ca\(^{2+}\) channels participate in ANF secretion. However, ANF release is more sensitive to mibefradil, especially during a weak depolarization activating mostly T-type channels, indicating their substantial contribution to ANF secretion. In addition, mibefradil inhibits a basal component of ANF secretion, which might be explained by the existence of the window current reflecting a population of T-type channels open within the range of the cells’ resting potential. Because of the predominance of T-type versus L-type Ca\(^{2+}\) channel expression in the early postnatal period, our data suggest an important contribution of cardiac T-type channels in Ca\(^{2+}\) signaling and in physiological functions such as hormone release during the neonatal period.

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REFERENCES