L-type but not T-type calcium current changes during postnatal development in rabbit sinoatrial node

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Protas, Lev, Dario DiFrancesco, and Richard B. Robinson. L-type but not T-type calcium current changes during postnatal development in rabbit sinoatrial node. Am J Physiol Heart Circ Physiol 281: H1252–H1259, 2001.—Although the neonatal sinus node beats at a faster rate than the adult, when a sodium current (INa) present in the newborn is blocked, the spontaneous rate is slower in neonatal myocytes than in adult myocytes. This suggests a possible functional substitution of INa by another current during development. We used ruptured [T-type calcium current (ICa,T)] and perforated [L-type calcium current (ICa,L)] patch clamps to study developmental changes in calcium currents in sinus node cells from adult and newborn rabbits. ICa,T density did not differ with age, and no significant differences were found in the voltage dependence of activation or inactivation. ICa,L density was lower in the adult than newborn (12.1 ± 1.4 vs. 17.6 ± 2.5 pA/pF, P = 0.049). However, activation and inactivation midpoints were shifted in opposite directions, reducing the potential contribution during late diastolic depolarization in the newborn (activation midpoints −17.3 ± 0.8 and −22.3 ± 1.4 mV in the newborn and adult, respectively, P = 0.001; inactivation midpoints −33.4 ± 1.4 and −28.3 ± 1.7 mV for the newborn and adult, respectively, P = 0.038). Recovery of ICa,L from inactivation was also slower in the newborn. The results suggest that a smaller but more negatively activating and rapidly recovering ICa,L in the adult sinus node may contribute to the enhanced impulse initiation at this age in the absence of INa.

Ca current; automaticity; development; diastolic depolarization

IN THE VENTRICLE, the expression of L-type (ICa,L) and T-type calcium current (ICa,T) changes during ontogenesis. In most mammalian species studied, the current density of ICa,L increases during development (10, 17, 24, 25, 32; see also 9). The results on ICa,T are not definitive, with studies (17, 31, 46) in the rat ventricle reporting a postnatal decrease in current density and studies in the rabbit ventricle finding either a developmental increase (44) or an absence of the current at all ages (36). In addition, Nuss and Marban (35) recorded strong ICa,T in newborn mice, whereas no current was found in fetal mice (10). There is no information about the developmental changes of calcium currents in sinus node cells (SNC). In the adult central sinus node, which has little or no functional fast sodium current (INa) (2, 11), ICa,L contributes not only to plateau potential as in the ventricle but also to action potential upstroke and perhaps late diastole (47). ICa,T is more pronounced than in ventricular myocytes and may participate in impulse initiation (13, 20). Because of the specific functional role of calcium currents in SNC, any developmental change in their biophysical characteristics could have significant impact on impulse initiation and automaticity. The importance of studying these changes is emphasized by the recent finding that SNC from newborn rabbits have a prominent TTX-sensitive fast INa (2). In that study (2), TTX markedly prolonged the cycle length of newborn cells but did not change it in adult cells, demonstrating that INa plays a key role in impulse initiation in the newborn sinus node. Additional data indicate that INa directly contributes to diastolic depolarization in newborn SNC (3). The observation that newborn (but not adult) SNC exhibited pronounced slowing of spontaneous rate after block of INa (2) suggests that, during development, another current (or currents) functionally substitutes for INa with respect to impulse initiation. The present study tested the hypothesis that ICa,L and/or ICa,T serve this purpose. Accordingly, one would expect an age-dependent increase in calcium current density and/or changes in biophysical characteristics resulting in increased contribution to impulse initiation. Unexpectedly, we did not find any developmental increase but rather a decrease in ICa,L current density with age. However, the activation curve of ICa,L was shifted negative in adult cells, whereas the inactivation curve was shifted positive; these opposite shifts should result in increased “window” current in adult cells compared with newborn cells. The wider window current, and in particular the more negative position of the activation curve, can increase inward current during late diastole and reduce the threshold potential for impulse initiation by ICa,L in adult SNC. An additional mechanism to in-

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increase the role of $I_{Ca,L}$ in action potential initiation may be the faster recovery from inactivation found in adult SNC in this study.

METHODS

SNC were prepared using a previously described method (12). Newborn (3–10 days old) and adult (41–48 days old) female rabbits were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (4.6 mg/kg) and then euthanized in accordance with protocols approved by the Animal Care and Use Committee of Columbia University. The heart was removed, and the sinus node was isolated and cut into four to six strips. Strips were rinsed for 5 min in a glass tube with a solution containing (in mM) 140 NaCl, 5.4 KCl, 0.5 MgCl$_2$, 1.2 KH$_2$PO$_4$, 5 HEPES-NaOH, 50 taurine, and 5.5 glucose (pH 6.9) and then triturated for 15–20 min in 5 ml of the same solution including enzymes and 200 $\mu$M CaCl$_2$. The amount of the following enzymes varied depending on the individual activity of samples and the age of the rabbits: 1.8–6 mg (600–2,000 units) collagenase (Worthington Biochem), 0.32–0.65 mg (1.5–3 units) protease (Sigma), and 0.05–0.10 mg (4.75–9.5 units) elastase (Sigma). Strips were rinsed for 5 min in K$^+$-rich solution (see composition in Ref. 47) and triturated in a fresh 5-ml volume of this solution to disperse cells mechanically. Both enzymatic and mechanical digestions were performed at 37°C. During the following 20 min, the concentrations of Na$^+$ and Ca$^{2+}$ were gradually increased while the concentration of K$^+$ was decreased to achieve final concentrations of 100, 1.3, and 35 mM, respectively. Cells were kept in this solution at 4–6°C and used in experiments 1–8 h after complete isolation.

During experiments, cells were placed in a heated (35°C) bath and superfused with a physiological saline solution of the following composition (in mM): 140 NaCl, 5.4 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 5 HEPES, and 10 glucose (pH 7.4). Only single, spindle-shaped, spontaneously beating cells were chosen for electrophysiological measurements. Membrane currents were recorded by the whole cell or perforated patch techniques using an IBM-compatible computer equipped with pCLAMP software (versions 6.0.3 or 8; Axon Instruments), a DigiData 1200 series interface (Axon Instruments), and an Axopatch 1C amplifier (Axon instruments). Borosilicate glass pipettes (Sutter Instrument) were filled with a solution of the following composition (in mM): 90 aspartic acid, 10 NaCl, 100 CsOH, 30 CsCl, 2 MgCl$_2$, 5 EGTA, 2 CaCl$_2$, 10 HEPES, 2 ATP Na$_2$, and 0.1 GTPNa$_2$; pH 7.2 (pCa = 7). Pipette resistance was 3–4 MΩ.

The whole cell configuration of the patch clamp was used to study $I_{Ca,T}$. After a seal was formed, the membrane was ruptured, and capacitance currents were compensated; the cell was superfused with a low-Na$^+$ solution of the following composition (in mM): 50 NaCl, 5 CsCl, 50 triethylammonium chloride, 0.5 MgCl$_2$, 10 HEPES, 2 CaCl$_2$, 5.5 glucose, and 0.01 TTX (pH 7.4). After $I_{Ca,L}$ rundown had ceased (see RESULTS), the current-voltage ($I$-$V$) relationship was determined from a holding potential of $-80$ mV using two protocols. The first protocol included a 50-ms step to $-50$ mV to inactivate $I_{Ca,T}$ and then 200-ms steps to potentials ranging from $-70$ to $50$ mV (increment, 10 mV). The second protocol was the same except that the step to $-50$ mV was omitted. $I_{Ca,L}$ was found by subtracting currents obtained with the first protocol from those obtained with the second one. Linear leakage and capacitative transients were corrected by proportional subtraction of mean current recorded during steps from $-80$ to $-60$ mV ($n = 10$ records).

To study the steady-state inactivation of $I_{Ca,T}$, the potential was held at $-40$ mV, and a 600-ms conditioning pulse (to voltages from $-100$ to $-40$ mV, 10-mV steps) was applied followed by a 100-ms pulse to $-30$ mV. In these experiments, 10 $\mu$M nifedipine (Sigma) was added to the control low-Na$^+$ solution to suppress $I_{Ca,L}$.

To study $I_{Ca,L}$, the perforated patch technique was employed by adding 100–200 $\mu$g/ml amphotericin B (Sigma) to the pipette solution. Two to ten minutes after the seal was formed, when series resistance was reduced to 15–20 MΩ, the normal superfuse was exchanged for one containing (in mM) 135 NaCl, 10 CsCl, 1.8 CaCl$_2$, 1 MgCl$_2$, 5 HEPES, 10 glucose, and 0.003 TTX. With the use of the perforated patch, the rundown of $I_{Ca,L}$ in either adult or newborn SNC was only 4% when checked every 5 s using 100-ms pulses to 20 mV from holding potential of $-50$ mV over a period of 4 min (data not shown). To construct $I$-$V$ curves, 200-ms steps ranging from $-45$ to $55$ mV were applied from a holding potential of $-50$ mV in the absence and in the presence of 10 $\mu$M nifedipine. $I_{Ca,L}$ was measured as the nifedipine-sensitive current.

The perforated patch and solutions described above were also used to study steady-state inactivation of $I_{Ca,L}$ and recovery of $I_{Ca,L}$ from inactivation. In this case, 100 $\mu$M NiCl$_2$ and 10 $\mu$M TTX were added to the cesium-containing solution. To study inactivation of $I_{Ca,L}$, we used a 1,000-ms conditioning pulse (to voltages from $-80$ to 10 mV, 10-mV steps) followed by a 2-ms step to $-50$ mV before the 300-ms test pulse to 10 mV. Holding potential was $-50$ mV. Currents were measured relative to the peak current at the test potential after the conditioning potential of $-80$ mV ($I_{max}$). Linear leakage and capacitative transients were eliminated by a standard subtraction method.

To study the recovery from inactivation, a two-pulse protocol was used. The holding potential was $-50$ mV; both pulses were from $-50$ to 20 mV and of 100-ms duration. The interval between the pulses was increased from 10 to 2,800 ms. The protocol was run twice in the absence and presence of nifedipine, and $I_{Ca,L}$ was measured as the nifedipine-sensitive current.

For the voltage-dependent activation and inactivation kinetics of $I_{Ca,T}$ and $I_{Ca,L}$, we assumed a single-exponential relation, as employed previously in the sinoatrial node (15, 20, 48). Activation and inactivation were analyzed according to Boltzmann fitting analysis, and recovery from inactivation was according to exponential analysis (Mircrocal Origin, version 6.0). Data are presented as mean ± SE. Statistical significance was determined by ANOVA or Student’s $t$-test as appropriate. A value of $P < 0.05$ was regarded as significant.

RESULTS

$T$-Type Calcium Current

Current density. After the patch was ruptured, the cell was adapted to the new conditions for several minutes, during which time the rundown of $I_{Ca,L}$ was estimated. The rundown of $I_{Ca,L}$ during whole cell patch-clamp recording is a well-known phenomenon (see, for example, Ref. 5). The rundown of $I_{Ca,T}$ was negligible or lacking (16, 34, 41). Moreover, Wetzel et al. (44) found in rabbit ventricular myocytes that with rundown of $I_{Ca,L}$, $I_{Ca,T}$ became more prominent. However, if $I_{Ca,L}$ rundown has not stabilized before the measurement of $I_{Ca,T}$ the value for $I_{Ca,T}$ found as a difference current may be distorted. To estimate the
dynamics of $I_{Ca,L}$ rundown, 200-ms pulses to 20 mV preceded by short steps to −50 mV from a holding potential of −80 mV were repeated every 5 s. Generally, the current stabilized in 2 min at −70% of the initial magnitude (data not shown). After this time, two $I$-$V$ protocols were run as described in METHODS. A typical result is shown in Fig. 1, which depicts the family of current traces with and without the conditioning step to −50 mV (Fig. 1, A and B) as well as the calculated difference current traces (offset for clarity; Fig. 1C). At both ages studied, the $I$-$V$ curves constructed from the difference current had a threshold between −60 and −50 mV and a peak at −20 mV (Fig. 2). The peak current in cells from newborn and adult animals was −4.4 ± 0.7 (n = 6) and −4.3 ± 0.7 pA/pF (n = 5), respectively. No significant difference between $I_{Ca,T}$ $I$-$V$ curves constructed for these two ages was found (two-way ANOVA, $P = 0.49$). Nickel, which is reported to preferentially inhibit $I_{Ca,T}$ in SNC (20), was used at a concentration of 100 μM and reduced peak current density at −20 mV by 80 ± 4% and 65 ± 9% in newborn and adult animals, respectively (data not shown). The $I$-$V$ data were used to construct activation curves for adult and newborn SNC.

**Activation of $I_{Ca,T}$**. Activation curves were constructed according to the equation $d(V) = g(V)/g_{\text{max}} = [I(V)_{\text{rev}} - V]/g_{\text{max}}$, where $d(V)$ is channel activation, $g(V)$ is conductance, $g_{\text{max}}$ is maximal conductance, $V_{\text{rev}}$ is the reversal potential, and $V$ is the test potential. The linear portion of the positive limb of the $I$-$V$ curve was extrapolated to obtain $V_{\text{rev}}$, which was 36 and 37 mV for newborn and adult SNC, respectively. As shown in Fig. 2B, the activation curves are similar at both ages, with no significant differences in either the midpoint ($V_{1/2}$) or inverse slope factor.

**Steady-state inactivation**. Steady-state inactivation of $I_{Ca,T}$ was expressed as $f(V) = I(V)/I_{\text{max}}$, where $I$ is peak current at the test potential after any given conditioning potential and $I_{\text{max}}$ is the peak current after a conditioning potential of −100 mV (Fig. 3). We found no developmental differences in the availability of $I_{Ca,T}$ ($V_{1/2}$: −65 ± 2.1 mV, n = 6, for newborn SNC and −65.1 ± 2.4 mV, n = 6, for adult SNC, $P = 0.943$; inverse slope factor: 9.7 ± 0.7 for newborn SNC and 10.5 ± 0.7 mV for adult SNC, $P = 0.464$). In both adult and newborn SNC, $I_{Ca,T}$ was inactivated by −20% at −80 mV (Fig. 3C), indicating that the current density in the $I$-$V$ relationship of $I_{Ca,T}$ in SNC held at −80 mV was slightly but equivalently underestimated at both ages.

![Fig. 1](http://ajpheart.physiology.org/) Experimental protocol used to record T-type calcium current ($I_{Ca,T}$). A and B: two sets of typical traces of Ca$^{2+}$ currents consecutively recorded from the same sinus node cell (SNC). A: currents obtained by stepping from a holding potential of −80 mV to the range of −70 to 50 mV. These currents contained both $I_{Ca,T}$ and L-type calcium current ($I_{Ca,L}$). B: currents obtained by the same steps after a 50-ms step to −50 mV to inactivate $I_{Ca,T}$. Corresponding wave form configurations are shown above each set of traces. C: difference current (defined as $I_{Ca,T}$) obtained by subtracting B from A; only the initial portion of current records are shown, with capacitance transients cropped, and every other trace was omitted for clarity. The transient current at the start of the prepulse to −50 mV (B) largely represents uncompensated capacitance, which has been corrected for in the difference traces of C.
L-Type Calcium Current

Current density. Representative current traces in the absence and presence of nifedipine (10 \( \mu \)M) and the calculated difference current are shown in Fig. 4, A–D, for an adult cell. The \( I-V \) relations of the current in the two age groups are shown in Fig. 4 E. Both curves were bell shaped but peaked at different voltages: approximately 0 mV for newborn and approximately 25 mV for adult SNC. Peak current density in newborn SNC (217.58 \( \pm \) 2.46 pA/pF, \( n = 15 \)) was significantly (\( P = 0.049 \)) greater than that in adult SNC (212.15 \( \pm \) 1.42 pA/pF, \( n = 8 \)). According to two-way ANOVA, the two \( I-V \) curves are significantly different (\( P < 0.01 \)).

Kinetics of decay. Figure 4F shows the voltage dependence of half-decay time (\( T_{1/2} \)) of \( I_{Ca,L} \) in newborn and adult SNC. The two curves have a similar shape, with the fastest \( T_{1/2} \) in the range of 20 to 0 mV. Two-way ANOVA revealed no significant difference (\( P = 0.739 \)) between the curves.

Activation of \( I_{Ca,L} \). For \( I_{Ca,L} \), \( V_{rev} \) was 55 mV for both newborn and adult. As shown in Fig. 5, the \( I_{Ca,L} \) activation curve for adult SNC was parallel to that for newborn SNC (inverse slope factor: 6.9 \( \pm \) 0.2, \( n = 8-10 \), and 7.3 \( \pm \) 0.1 mV, \( n = 14-15 \), respectively), but the curve for adult SNC was shifted to more negative potentials by \(-5 \) mV (\( V_{1/2} \): -22.3 \( \pm \) 1.4 mV in the adult and -17.3 \( \pm \) 0.8 mV in the newborn, \( P = 0.001 \)).

Steady-state inactivation. As apparent in Fig. 5, the threshold for inactivation was approximately \(-60 \) mV for both the newborn and adult; at \(-40 \) mV, 30% and 15% of \( I_{Ca,L} \) was inactivated in newborn and adult SNC, respectively. Boltzmann curves best fitting the data from newborn and adult SNC were parallel (inverse slope factors: 6.6 \( \pm \) 0.3, \( n = 7 \), and 7.1 \( \pm \) 0.4 mV, \( n = 7 \), respectively) but contrary to the results on activation, the curve for adult SNC shifted to more positive potentials; the shift was significant (\( V_{1/2} \): \(-33.4 \pm 1.4 \) and \(-28.3 \pm 1.7 \) for newborn and adult SNC, respectively, \( P = 0.038 \)).
Recovery from inactivation. The results of these experiments are presented in Fig. 6, with individual current traces for a 10-day-old SNC shown in Fig. 6A and the mean time dependence of recovery plotted in Fig. 6B. In newborn SNC, \( I_{\text{Ca,L}} \) recovered more slowly than in adult SNC. The time course of recovery was best described by a biexponential function, the time constant \( \tau \) for the fast component being significantly different \((112.2 \pm 13.4 \text{ ms}, n = 6, \text{ and } 63.1 \pm 8.5 \text{ ms}, n = 5, \text{ for newborn and adult SNC, respectively, } P = 0.017)\). \( \tau \) of the slow component also tended to be larger in newborn than adult SNC \((2,538 \pm 1,476 \text{ vs. } 958 \pm 254 \text{ ms})\), but the difference was not significant \((P = 0.363)\).

DISCUSSION

We studied the functional expression of two types of calcium channels in SNC to assess their possible role in action potential generation in young and adult hearts. The results show no differences in current density, activation, or availability of \( I_{\text{Ca,T}} \) in newborn SNC compared with adult SNC. On the contrary, significant developmental changes were found in \( I_{\text{Ca,L}} \).

In this study, \( I_{\text{Ca,T}} \) was identified as a difference between current containing both \( I_{\text{Ca,T}} \) and \( I_{\text{Ca,L}} \) and current containing only \( I_{\text{Ca,L}} \). As expected for a specific dissection of the \( I_{\text{Ca,T}} \) component, the difference current had fast activation and decay and sensitivity to nickel. As previously mentioned, adult rabbit SNC have very little or no fast \( I_{\text{Na}} \) \((2)\). The current identified as \( I_{\text{Ca,T}} \) was not the TTX-sensitive \( I_{\text{Na}} \) expressed in newborn SNC, because 1) the difference current in newborn SNC had the same properties as in adult SNC, 2) \( 100 \mu M \) nickel does not inhibit the fast \( I_{\text{Na}} \) of newborn SNC \((2)\), and 3) the superfused solution contained reduced \( Na^+ \) and \( 30 \mu M \) TTX, a concentration three orders of magnitude greater than the IC\(_{50}\) for \( I_{\text{Na}} \) block in newborn SNC \((26 \text{ nM}) \((4)\).

In our study, no significant age-related differences were found in \( I_{\text{Ca,T}} \) density, activation, and availability. These results differ from those in several studies of rabbit ventricular myocytes. In one study \((36)\), no \( I_{\text{Ca,T}} \) was found in both newborn and adult rabbit ventricular myocytes. In another study \((44)\), \( I_{\text{Ca,T}} \) was detected and reduced in neonatal compared with adult cells; however, a detailed analysis of activation and inactivation relations was not included. In the case of rabbit SNC, further studies are required to determine which subunits of the T-type calcium channel are expressed developmentally. It was recently shown \((6)\) that the adult mouse sinus node has transcripts for two T-type calcium channel isoforms, Cav3.1 and Cav3.2 \((\alpha_{3G}-\text{ and } \alpha_{1H}-\text{subunits, respectively; see Ref. 23})\), with a dominant expression of Cav3.1. The pattern of expression of

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**Fig. 5.** Effect of age on activation and availability of \( I_{\text{Ca,L}} \). Activation \( (d_{\text{f}}) \) and steady-state inactivation \( (f_{\infty}) \) curves for \( I_{\text{Ca,L}} \) and their Boltzmann fits (lines) in newborn and adult SNC are shown. The activation curve for adult SNC shifted negative, whereas the inactivation curve shifted positive, relative to the corresponding curves for newborn SNC, resulting in a wider “window” current in adult SNC. \( V_{50} \) for both activation and inactivation are significantly different between the two age groups (see text).

**Fig. 6.** Effect of age on recovery from inactivation of \( I_{\text{Ca,L}} \). A: two-pulse protocol and typical traces of nifedipine-sensitive current for a 10-day-old SNC. Only the first 7 of 12 traces are shown. B: time dependence of recovery from inactivation. Curves were fit by a biexponential function (lines), and the time constant for the faster component was significantly different (see text). Inset, expansion of early portion of curve.
the isoforms in the newborn sinus node is not known. However, the two isoforms do differ in their sensitivity to nickel when tested in heterologous expression systems, with Cav3.2 being the more sensitive (29). Given that we did not observe a significant difference either in the percent block by 100 μM nickel or any biophysical difference in current characteristics in newborn SNC versus adult SNC, there is no reason to propose the presence of a different isoform expression pattern in the newborn.

The relatively large density of $I_{\text{Ca,T}}$ in adult SNC and the sensitivity of SNC action potentials to nickel (which blocks $I_{\text{Ca,T}}$ in SNC) led to the suggestion that $I_{\text{Ca,T}}$ contributes to the later two-thirds of diastolic depolarization and plays an important role in impulse initiation in SNC (20). The present study did not directly address this issue. However, the observation that $I_{\text{Ca,T}}$ is functionally mature in the newborn SNC and yet cells at this age exhibit slow or no spontaneous rate in the absence of $I_{\text{Na}}$ (2) suggests that $I_{\text{Ca,T}}$ is not sufficient to assure normal impulse initiation. The lack of developmental changes in $I_{\text{Ca,T}}$ found in our experiments also makes $I_{\text{Ca,T}}$ an unlikely candidate as a current substituting for $I_{\text{Na}}$ in the adult SNC. In terms of the specific biophysical characteristics of $I_{\text{Ca,T}}$ in SNC, the activation data reported here agree with those reported by two other groups (20, 30). The inactivation relation in our study was more shallow than that reported by Hagiwara et al. (20) but comparable with that of Lei et al. (30).

The following differences in $I_{\text{Ca,L}}$ were found in this study for adult SNC versus newborn SNC: 1) current density decreased, 2) activation curve shifted to the left, 3) steady-state inactivation (availability) curve shifted to the right, and 4) recovery from inactivation became faster. Despite the greater current density in the newborn, there was no difference in the time course of inactivation. Because this time course includes a calcium-dependent component, this suggests that the local calcium concentration at the inner surface of the channel may not differ with age. This might argue against either a greater calcium influx per channel in the neonate or a significant channel clustering. However, one should remember that studies (21, 39, 40) in adult SNC have provided evidence of a contribution of intracellular calcium regulation to automaticity and that there is evidence in the ventricle (but not yet in the sinus node) for developmental regulation of calcium regulatory mechanisms (18). Therefore, the possibility of developmental differences in calcium homeostasis and/or its contribution to automaticity in SNC merits future consideration.

The opposite developmental shift in activation and inactivation curves results in a wider window current in adult SNC, potentially providing more current in the voltage range of diastolic depolarization. However, this is opposed by a smaller current density in adult SNC. To estimate the current available at window voltages, we calculated $I_{\text{Ca,L}}$ density multiplied by corresponding values of $I_{\text{Ca,L}}$ availability (Fig. 7). This current represents the size of $I_{\text{Ca,L}}$ that would be available in steady-state conditions. Available $I_{\text{Ca,L}}$ was greater in adult SNC than in newborn SNC at voltages from −45 to −5 mV. Voltages corresponding to the diastolic depolarization range (−50 to −35 mV) are of particular interest. At −40 mV, the calculated available current in adult SNC was 1.9 times that in newborn SNC (−1.38 and −0.74 pA/pF, respectively). Verheijck et al. (42) suggested that $I_{\text{Ca,L}}$ activates at more negative potentials than those reported here in adult SNC and thus could contribute to the entire range of diastolic depolarization.

In agreement with our results on rabbit SNC, Wetzel et al. (45) have shown that, in adult rabbit ventricular myocytes, $I_{\text{Ca,L}}$ inactivates at more positive potentials than in fetal and neonatal myocytes, resulting in a wider window current and suggesting that increasing availability of $I_{\text{Ca,L}}$ may be a common feature of developing rabbit cardiac cells. On the other hand, Osaka and Joyner (36) found no difference in activation or inactivation of $I_{\text{Ca,L}}$ in ventricular myocytes in newborn versus adult rabbits.

In addition to steady-state inactivation and the threshold of activation, the kinetics of recovery from inactivation may further limit the contribution of $I_{\text{Ca,L}}$ to impulse initiation in newborn SNC. We found that, in adult SNC, $I_{\text{Ca,L}}$ recovers significantly faster than in newborn SNC ($\tau$ of the fast component of recovery was $63.1 \pm 8.5$ ms in adult SNC vs. $112 \pm 13.4$ ms in newborn SNC). $I_{\text{Ca,L}}$ can effectively recover from inactivation beginning with the end of action potential repolarization. On the basis of a study (22) in newborn and adult intact sinus nodes of rabbits, the time from the end of repolarization to the point at which diastolic depolarization reaches action potential threshold can be estimated to be 150 ms at physiological temperature. If recovery kinetics during diastolic depolarization mirror the data shown in Fig. 6 (which is based on a fixed potential of −50 mV), then one would predict that $I_{\text{Ca,L}}$ recovers to 60% and 75% of maximal values in newborn and adult SNC, respectively. Thus, in the newborn, the negative shift of the steady-state inactivation relation (Fig. 5) combined with the slower re-

![Fig. 7. $I_{\text{Ca,L}}$ available at steady-state conditions (window current) in newborn and adult SNC. Values were found as a product of availability of $I_{\text{Ca,L}}$ (see Fig. 5) and current density (see Fig. 4E) at the same voltages.](http://ajpheart.physiology.org/...
covery from inactivation in the newborn are likely to result in less \( I_{Ca,L} \) available in late diastole, whereas the positive shift of the activation relation will require depolarization to less negative voltages to achieve threshold for \( I_{Ca,L} \) activation.

There are several possible explanations for the observed changes in parameters of \( I_{Ca,L} \) with development. There could be an age-dependent isoform switch of one or more channel subunits. Four isoforms have been described for the pore-forming \( \alpha_1 \) subunit of L-type calcium channels, among them \( \alpha_{1C} \) (cardiac form) and \( \alpha_{1D} \) (neuroendocrine form) (23) as well as splice variants (14). Bohn et al. (6) demonstrated that the adult mouse sinus node has clearly detectable mRNAs for Cav1.2 (\( \alpha_{1C} \)-subunit) as well as a low level of Cav1.3 (\( \alpha_{1D} \)-subunit). As noted by these authors, Cav1.3 may be of functional importance in SNC because mice lacking \( \alpha_{1D} \) have abnormalities in sinus rhythm (38). If rabbit SNC also have more than one type of \( \alpha_1 \)-subunit, it would be important to assess the relative prevalence of the isoforms as a function of development. One also must consider alterations in the relative abundance or isoform expression of other channel subunits. Furthermore, changes in membrane localization and/or cytoskeletal interaction are reported to impact on the functional expression of currents, including \( Na^+ \) (8), \( K^+ \) (7, 33), and \( Ca^{2+} \) currents (28, 37).

In addition, changes in calcium channel function may be secondary to the developmental changes of other calcium signaling components and/or their interaction with calcium channels. Recently, it has been shown that inhibition of \( Ca^{2+}/calmodulin \)-dependent protein kinase II (CaMKII) in adult rabbit SNC shifted the \( I_{Ca,L} \) availability curve negative by 11 mV (43) and significantly prolonged recovery from inactivation. The data suggest the existence of strong control of steady-state inactivation of \( I_{Ca,L} \) by CaMKII. In the rat heart, the predominant expression of the functionally important \( \delta \)-subunit of CaMKII switches from the \( \delta_1 \)-isoform in fetal and neonatal hearts to the \( \delta_2 \)-isoform in adult hearts (19), which, in turn, can provide a basis for functional modulation of calcium current. Furthermore, other signaling cascades that regulate the basal phosphorylation level of calcium channels are known to be subject to developmental changes in the rabbit ventricle (26, 27), and a similar age-dependent regulation could occur in the sinus node. In this regard, it is worth remembering that \( I_{Ca,L} \) was measured with the perforated patch technique. Thus the intracellular environment was minimally disrupted, and measured differences in ionic currents could in part be secondary to differences in the intracellular environment (e.g., divalent cation concentration) as a function of age.

In conclusion, we found significant developmental changes in \( I_{Ca,L} \) but not \( I_{Ca,T} \) in rabbit SNC that could contribute to the deficiencies in neonatal automaticity after block of \( I_{Na} \). There are changes in activation, steady-state inactivation, and recovery from inactivation of \( I_{Ca,L} \), resulting in the likely higher availability of this current in adult SNC during late diastole at an age when \( I_{Na} \) no longer significantly contributes to SNC diastolic depolarization (3) or impulse initiation. Finally, it should be noted that this does not rule out additional differences in other currents in the sinus node as a function of age. A previous study (1) reported that the pacemaker current exhibited greater current density with no change in the voltage dependence in the neonate, thus arguing against a deficiency of that current in the newborn. However, the role of developmental changes in potassium currents in the sinus node remains unexplored. In addition, it is known that the adult sinoatrial node exhibits regional heterogeneity with respect to ion channel expression and function (48); the extent of heterogeneity within the neonatal sinoatrial node has not been fully explored.

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REFERENCES


