Electrophysiology and pacemaker function of the developing sinoatrial node

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Baruscotti M, Robinson RB. Electrophysiology and pacemaker function of the developing sinoatrial node. Am J Physiol Heart Circ Physiol 293: H2613–H2623, 2007. First published September 7, 2007; doi:10.1152/ajpheart.00750.2007.—The sinoatrial node performs its task as a cardiac impulse generator throughout the life of the organism, but this important function is not a constant. Rather, there are significant developmental changes in the expression and function of ion channels and other cellular elements, which lead to a postnatal slowing of heart rate and may be crucial to the reliable functioning of the node during maturation. In this review, we provide an overview of current knowledge regarding these changes, with the main focus placed on maturation of the ion channel expression profile. Studies on Na+ and pacemaker currents have shown that their contribution to automaticity is greater in the newborn than in the adult, but this age-dependent decrease is at least partially opposed by an increased contribution of L-type Ca2+ current. Whereas information regarding age-dependent changes in other transmembrane currents within the sinoatrial node are lacking, there are data on other relevant parameters. These include an increase in the nodal content of fibroblasts and in the area of connexin43, considered a molecular marker of nodal tissue. Although much remains to be done before a comprehensive view of the developmental biology of the node is available, important evidence in support of a molecular interpretation of developmental slowing of the intrinsic sinoatrial rate is beginning to emerge.

cardiac pacemaker; development; sodium current; calcium current

THE ANATOMICAL IDENTIFICATION of the sinoauricular junction [now named the sinoatrial node (SAN)] was originally reported in 1907 by two physiologists/anatomists, Keith and Flack (58). This finding, together with the earlier identification of other structures of the cardiac conduction system (104, 110, 123), prompted further studies (70, 124) that soon yielded the concept that the heartbeat in mammals originates in the SAN and then spreads to the rest of the cardiac tissue in a temporally and anatomically orderly manner. The impact on the cardiac scientific community was immediate and led to continuing effort to unravel the working mechanisms of this biological impulse generator. As a result, automaticity mechanisms in the adult SAN have been extensively studied and have been the subject of numerous reviews (21, 31, 48, 98, 99, 120). Despite this, the basis of automaticity, and the issue of the relative importance of different currents to pacemaking, remains controversial. However, a few generalities can be stated. Pacemaking will depend on current flow during diastole as well as the presence and characteristics of the major depolarizing currents that take the cell to and beyond threshold. Multiple currents are likely to contribute to both diastolic depolarization and threshold, and the mix varies with species, with disease, and regionally within the node. In addition to the characteristics of the channels at the cellular level, pacemaking also will be influenced by characteristics of the tissue, including connections to surrounding atrial myocytes and fibroblasts.

Despite the extensive literature devoted to the ionic basis of automaticity in the adult SAN, and the fact that age-dependent differences in intrinsic heart rate have been recognized for some time (52, 53, 87, 114, 127, 128), there has been far less research on the basis of automaticity in the newborn or immature node. There is, however, sufficient evidence to state categorically that the ionic basis of automaticity in the newborn is distinct from that of the adult. These differences can have important implications for the diagnosis and treatment of cardiac rhythm disorders in the immature, adult, and aged individual. In this review, we focus specifically on the young versus adult SAN but include data on the aged heart where appropriate. We do not describe the adult characteristics in detail but do refer to published reviews and simply highlight key aspects for the purpose of comparison to the young node. Our intent is, in part, to educate the reader to the fact that the basis of SAN automaticity is quantitatively and qualitatively distinct in the newborn and adult. However, our additional purpose is to highlight how much remains to be determined in this field and thereby to encourage additional research on the topic.

General Properties of the SAN

In mammals, the pacemaker region of the heart (the SAN) is located in the wall of the right atrium, but its precise location can vary slightly in different species. In rabbits, a long-standing model for heart automaticity studies, it extends along
Invited Review

Molecular Markers of the SAN

Until recently, the only way to precisely identify the SAN region was electrical mapping (26, 126). This technique continues to be utilized in basic and clinical research since it can be applied to both in vivo and in vitro preparations and permits a precise description of the progression of the depolarizing wave. More recently, molecular-based identifications have been suggested, based on comparative studies of adult sino-atrial and atrial myocytes that rely on the presence or absence of specific molecular markers. Combined efforts of several groups have determined that adult SAN cells express connexin (Cx)45 and neurofilament M (NF-M) but do not express Cx43 and atrial natriuretic peptide. This expression profile is completely reversed in atrial cells. Furthermore, crucial differences also exist at the level of ionic currents; for example, SAN cells are characterized by the pronounced expression of pacemaker current (Ih; see Ionic Channels in the Developing Node), whereas they express little or no inwardly rectifying K+ current (24, 32, 48, 85). These data have been largely collected in adult preparations; therefore, an important question that should be asked is whether the presence or absence of these molecular markers is stable during the entire lifetime of the organism. If not, this would raise the possibility that variations in the expression of these proteins could be linked to selective and specific age-dependent functional differences in the node that could eventually lead to the occurrence of aging-related pathologies. Furthermore, a strong developmental regulation of any of these proteins would impact their utility as markers in the young or perhaps aged SAN.

Expression of NF-M. Studies (39, 121) have explored the expression of NF-M in the conduction system of the adult heart as well as during embryonic development in rabbits. Positive staining for NF-M is considered an appropriate hallmark for the identification of this specialized tissue (33, 35). NF-M can be considered an early marker of the developing conduction system of the embryo since it is already detectable at embryonic day 9.5 (E9.5) just before heart looping has completed (121), and its expression profile is complete by birth (40). However, in considering the relevance of NF-M as a tool to identify and investigate the properties of the pacemaker region, it is important to note that there may be marked species differences, since there has been no evidence for the presence of NF-M in humans (121) and rats (5).

Expression of connexin isoforms. Connexins can serve both as molecular markers and as important contributors to the electrophysiological properties of the node. Connexins are proteins that, when properly assembled, give rise to gap junctions that allow the intercellular exchange of ions and small molecules. In the human and mouse genome, the family of connexins includes at least 20 members (106). For several years the isoforms known to be expressed in cardiac tissue were Cx40, Cx43, and Cx45. This list has been recently updated by Kreuzberg et al. (64), who also reported the presence of Cx30.2. The distribution of these isoforms is not uniform throughout all heart regions. Indeed, Cx40 and Cx43 are mostly present in working atrial and ventricular myocytes, whereas conduction tissue is characterized by the presence of Cx45 and Cx30.2 (the mouse homolog of human Cx31.9). In particular, Cx30.2 seems to be selectively expressed in the SAN and atrioventricular (AV) node (64). This pattern of expression has been particularly useful in studies of the SAN since the absence of Cx43, originally observed in several studies (28, 29, 86, 112, 116), has been utilized to identify the SAN region from the surrounding Cx43-rich atrial tissue. In terms of functionality, Cx45 has a somewhat lower unitary conductance than either Cx40 or Cx43 (17, 18). Furthermore, Cx30.2 has the lowest unitary conductance of all gap junctional proteins, and knocking out this connexin results in a 25% decrease in the PQ interval (63). Thus, the nodal-specific connexin expression pattern may be critical to its property of slow conduction.

Jones et al. (52) investigated the expression profile of Cx43 in the SAN throughout the lifetime of the guinea pig. They found Cx43 to be uniformly present throughout the node in newborn animals, but progression from youth to senescence was accompanied by a 14-fold increase in the nodal area.
lacking Cx43 and a corresponding decrease in the level of Cx43 protein expression (Fig. 1). Interestingly, and in agreement with previous data, the disappearance of Cx43 initiated in the central nodal area and then extended to more peripheral nodal regions with further aging. Furthermore, there were no increases in Cx40 and Cx45 to compensate for the loss of Cx43. Two key questions regarding this example of developmental regulation of Cx43 are 1) what is its influence on functional aspects of the pacemaker site (i.e., does the disappearance of Cx43 relate to the intrinsic heart rate and/or nodal propagation)? and 2) what are the modulatory signals that control Cx43 expression? While the latter is still unanswered, some work has been done regarding the former. In particular, Yamamoto et al. (126) reported that the nodal area in which Cx43 is absent is characterized by a fivefold decrease in conduction velocity, and this is consistent with the concept that a dependence solely on Cx45 and Cx3.8 would lead to slower conduction. Furthermore, Jones et al. (52) observed that the distance that the impulse generated at the center of the node must travel before reaching atrial tissue (identified by the crista terminalis) increases with age. Taken together, these findings provide a molecular correlation to the functional observation of a decrease in the overall intrinsic heart rate and conduction time with age.

Fibroblast content of the developing node. The fibroblast component is one of many factors differentiating SAN structure from that of other cardiac regions. For example, in humans, the fibroblast content of the adult working myocardium is ~5–6% of the total volume, but this proportion is much higher in the SAN, where it reaches values of 45–75% (19, 27, 103). Studies (3, 19, 27, 103) on humans and lower mammals also have highlighted that the fibroblast, and therefore collagen, content of the heart is subject to strong developmental regulation: it is low during initial embryogenesis and increases during late embryogenesis and postnatal growth. After this period of expansion, the proliferation halts but can be triggered by the occurrence of pathological states. The apparent lack of a symmetrical organization of the myocardial component of the node is also true for the fibroblasts since they are found both adjacent to (and thus in contact with) sinoatrial myocytes and as isolated groups (20). It is interesting to note that these two separate populations also display different types of connexins. Fibroblasts interacting with nodal cells preferentially express Cx45, whereas those that do not contact SAN cells express Cx40; Cx43 has not been found in sinoatrial node fibroblasts (20). The contribution of the fibroblast population to the physiology of the node is complex, but important roles certainly include mechanical support and regulation of local inflammation (105). Since fibroblasts are nonexcitable, their dense presence in the SAN, and the observation that they are at least partially coupled via functional gap junctions to nodal cells, pose the intriguing question of a possible electrical consequence. In this regard, an interesting observation is that fibroblast resting potential is influenced by stretch as a direct consequence of the presence of stretch-activated ion channels on their cell membrane (56, 60). Whether changes in the membrane potential of fibroblasts directly influence the chronotropicism of coupled nodal cells is an interesting question that awaits investigation.

In addition to the observations above concerning fibroblast distribution during early development, changes in the structural properties of the SAN associated with aging result in slow conduction and reduced voltage amplitude on electroanatomic mapping (59, 95). Interestingly, these functional changes display some similarities with those observed in pathological states such as SAN dysfunction (43, 59, 95). Finally, besides their above-mentioned roles, it is possible that fibroblasts contribute to the metabolic homeostasis of the node (19).

**Ionic Channels in the Developing Node**

$I_f$. Pacemaker cells lack a stable resting potential, instead exhibiting a slowly rising depolarization that encompasses the interval from the end of action potential repolarization to the upstroke of the subsequent action potential. The events underlying this diastolic depolarization are the main determinants of cardiac pacemaker rate. Since its early discovery, $I_f$ has been identified as a contributor to diastolic depolarization (15), and, indeed, its activation upon hyperpolarization and its inward and time-dependent $Na^\%/K^+$ mixed nature are well suited to drive cell depolarization during the diastolic interval. The vast majority of information on the biophysical and molecular properties of this current derives from studies in single SAN myocytes isolated from adult animals (6, 25, 31, 93, 108). In addition to its contribution to basal pacemaking, $I_f$ is a preferential target in the autonomic modulation of cardiac chronotropism due to the presence of a CAMP-binding domain on the channel’s COOH terminus. The channels responsible for $I_f$ (f channels) are modulated by both sympathetic and parasympathetic branches of the autonomic nervous system; activation
of the muscarinic cascade affects f channels by reducing cAMP bound to the channel, thereby inducing a hyperpolarizing shift of the activation curve, whereas the stimulation of β-adrenoceptors elicits an increase in cAMP available for binding to the channel and thus increases I_{f} during diastole due to a depolarizing shift of the activation curve. To assess whether I_{f} undergoes developmental changes that could at least partially explain the slower heart rate observed in adult animals, Accili et al. (2) carried out comparative experiments of I_{f} in SAN myocytes isolated from newborn (9–10 day old) and adult rabbits. They found that newborn SAN cells had both a greater I_{f} density (newborn: 0.244 pS/pF and adult: 0.158 pS/pF) and a significantly steeper slope of the activation curve (slope factor: −9.6 mV in the newborn and −11.3 mV in the adult) compared with adult cells but no differences in the midpoint of activation (newborn: −66.7 mV and adult: −66.3 mV). These results, in addition to the observation that the capacitance of SAN cells does not vary with development (7), indicate that the amount of I_{f} that flows at pacemaker potentials is greater in newborn animals than in adults and thus could contribute to the higher intrinsic heart rate of the rabbit newborn SAN (114).

The intrinsic variation of the SAN rate during development has also been studied by Yang et al. (127, 128). These authors carried out radioimmunoassay experiments to evaluate age-dependent changes in the basal concentration of cAMP in sinoatrial cells and reported a marked decrease (0.31 μM at 2 wk vs. 0.025 μM at 12 wk). They also reported a negative shift of the I_{f} threshold potential (−45.5 vs. −51.1 mV in 2- vs. 4-wk-old hearts, respectively). Based on these findings, the authors proposed that heart rate slowing during development can be partially attributed to a reduced contribution of I_{f} secondary to a decrease in cellular cAMP content. The observation that cAMP levels change during development is interesting, but any extrapolation from these data must be done with caution since there is increasing evidence that membrane proteins such as ion channels exist in highly controlled microdomains where the concentration of cytoplasmic molecules such as cAMP can be different from those of the bulk cytoplasm. Furthermore, the data of Yang et al. (127, 128) differ from those of Accili et al. (2), who reported no differences in either the midpoint or acetylcholine- and isoproterenol-induced shifts of I_{f} activation curves between newborn and adult animals. Although Accili et al. (2) did not observe an age-dependent change in the cAMP responsiveness of f channels, this does not preclude an age-dependent difference in the modulation of I_{f} or heart rate. The developmental differences in the biophysical properties of the channels (higher conductance and steeper activation relation in the newborn) means that an identical shift in the position of the activation curve would per se lead to a greater current variation and thus to a more marked effect of autonomic control of heart rate in the newborn, as indeed has been observed both for vagal (30) and β-adrenergic (44) stimulation. This is illustrated in Fig. 2, where the activation parameters of Accili et al. (2) have been used to plot the relative activation relations of newborn and adult SAN cells under basal conditions and under the influence of high concentrations of acetylcholine and isoproterenol to represent maximal vagal and sympathetic tone, respectively. As shown, at a potential of −55 mV, which is the approximate maximum diastolic potential in the SAN, the amount of current contributed by I_{f} under maximal vagal tone is approximately the same in the newborn and adult. However, with maximal sympathetic tone, I_{f} increases only 2.8-fold in the adult compared with 4.5-fold in the newborn, representing a 60% greater change in I_{f} at the younger age.

Molecular identification of pacemaker channels, either at the message or protein level, has been carried out in several species, including rabbits, mice, rats, and dogs (38, 46, 49, 71–73, 96, 97, 100, 102, 115, 135). There are convincing indications that in the adult SAN, each functional channel is composed of four subunits belonging to the family of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. The major component of the native SAN I_{f} is generally the HCN4 isoform (49, 71, 79, 81, 102), although a recent study (46) using laser capture and RT-PCR unexpectedly found that in the rat SAN, the major isoform at the message level is HCN2. While these data on transcript expression need to be extended to the protein level, this same study also reported changes in message as a function of age. Both HCN2 and HCN4 transcript expression progressively decreased with development, and, consistent with this observation, the nonselective f channel blocker cesium became less effective in slowing rate with development (46). To our knowledge, this is the only study of I_{f} in the developing rat SAN, and so the results await confirmation by other groups and extension to other species.

The presence of HCN4 signal is generally considered a marker for the early identification of the SAN region during embryonic development (80). Although when singularly expressed in a heterologous system all members of the HCN family display similar properties, the most distinguishing char-

**Fig. 2.** The range of pacemaker current (I_{f}) availability under autonomic control is greater in the newborn than in the adult. Activation curves of I_{f} obtained in control and in the presence of maximal sympathetic (isoproterenol, ISO) or vagal (acetylcholine, ACH) activation are shown. The range of fractional activation at −55 mV, a voltage near the maximal diastolic potential, is 60% greater in the newborn than in the adult. The parameters of the activation curves were taken from Accili et al. (2).
characteristic of HCN4 is its slow kinetics (typical time constants of activation at −95 mV are 0.11, 1.13, and 2.52 s for HCN1, HCN2, and HCN4, respectively) (1, 4). These kinetics can be modified by specific interactions with physiological modulators such as phosphoinositol (4,5)-bisphosphate or MinK-related protein 1, which profoundly increase the current density and speed up the activation process of some HCN isoforms (89, 92, 131). Although to our knowledge no specific data are available on the molecular identification of the newborn I_{f} in the rabbit heart, the observation by Accili et al. (2), that its properties are similar to those of adult animals, would tend to suggest a similar molecular assembly. Further evidence in support of a prevalent HCN4 composition of newborn pacemaker channels comes from embryological data in mice. In a pivotal study, Stieber et al. (107) generated both global and cardiac-specific HCN4 knockout mice and showed that these mice die at about E9.5–E11.5 due to severe functional anomalies of the cardiac conduction system. Analysis carried out in this period confirmed that mice lacking HCN4 were characterized by a severe bradycardia (88 beats/min for HCN4−/− mice vs. 139 beats/min for wild-type HCN4+/+ mice), likely caused by a 75–90% reduction in I_{f}. Interestingly, the cardiac rate of knockout mice could not be modulated by a cAMP-dependent mechanism, thus strengthening the evidence that cAMP-dependent rate modulation is mostly induced by an I_{f}-dependent mechanism. Given the molecular composition and biophysical properties of I_{f} during embryological and postnatal development, it is reasonable to conclude that HCN4 subunits are central elements of cardiac pacemaker myocytes at all ages. Thus, an important aspect of future studies will be the analysis of the genetic mechanisms that regionally and temporally control or repress HCN4 channel expression. In this respect, Kuratomi et al. (65) recently investigated some aspects of the molecular mechanism controlling the expression of HCN4 and identified both the promoter region responsible for basal activity and the presence within the first intron of a conserved neuron-restrictive silencer element (NRSE) sequence. NRSE is a sequence that, when bound to neuron-restrictive silencing factor (NRSF), inhibits the transcriptional processing of neural genes in non-neuronal cells. Interestingly, Kuratomi et al. (65) found that the NRSE-NRSF system plays a crucial role in controlling the known progressive decrease of HCN4 channels during embryological development of the working myocardiun (129). Indeed, the levels of NRSF signal increase during embryological development, thus leading to a substantial inhibition of the transcriptional activity of the HCN4 gene. It is worth noting that the same mechanism operating in the opposite direction controls the increased expression of HCN4 channels induced by cardiac hypertrophy (36, 65, 66). Finally, the role of the NRSF-NRSE system in controlling channel expression is not limited to HCN4 but is also believed to control transcription of T-type Ca^{2+} channels (66, 130). The extent to which these regulatory mechanisms are active in the developing SAN remains to be determined.

Information on the mechanism controlling the differential fate determination of cardiac embryonic cells bound to become SAN or atrial cells is also currently becoming available. As shown by Hoogaars et al. (45), the transcriptional repressor Tbx3 seems to be the key element inducing the formation of the SAN from the rest of the (Tbx3-free) future working atrial myocardium. Whether or not the presence of Tbx3 is directly involved in the expression of HCN4 channels is not known. However, it is likely that the elucidation of the molecular events controlling the expression of the pacemaker phenotype will have practical implications for understanding arrhythmogenic cardiac diseases and innovative therapeutic strategies.

Na⁺ currents. Until a decade ago, it was commonly accepted that there was little or no functional contribution of Na⁺ current (I_{Na}) to the activity of primary sinoatrial pacemaker cells. This conclusion was mostly derived from the observation that the Na⁺ channel blocker TTX did not affect the spontaneous rate of either the intact SAN (61, 62, 69, 125) or single cells from adult rabbits (82, 83). In more recent years, the view that I_{Na} is irrelevant to pacemaker activity has been profoundly modified by developmental studies in the rabbit and by studies of the adult SAN in other species (7, 24, 34, 68, 75).

In 1996, we (7) reported that an I_{Na} was present in rabbit primary nodal cells at birth. Its developmental regulation was evident from the observation that by the 40th postnatal day, the current had fully disappeared (7). The functional role of the current was assessed by challenging the spontaneous activity of newborn cells with the Na⁺ channel blocker TTX (3 μM). Somewhat unexpectedly, TTX modified all action potential parameters rather than only the upstroke velocity. Particularly intriguing was the decrease in the slope of the diastolic depolarization from 0.035 to 0.015 V/s and the consequent slowing of spontaneous rate from 90 to 33 beats/min. Similar investigations of adult SAN cells failed to demonstrate I_{Na}, and, as expected, TTX did not affect rate. This marked age-dependent difference in the effect of TTX on spontaneous rate is shown in Fig. 3. Whole cell and single channel kinetic analyses demonstrated that a slow inactivation process due to multiple single channel reopenings at pacemaker potentials was the cause for the small but significant inward current that, in addition to I_{f}, contributed to diastolic depolarization (8, 9). The contribution of this current to diastolic depolarization is best seen under ramp-clamp conditions, where the membrane potential is slowly depolarized and the net current recorded. When TTX is used to block the contribution of any I_{Na}, one can determine the TTX-difference current. As shown in Fig. 4, A and B, there is a marked TTX-sensitive inward current during this membrane depolarization in a newborn SAN but not in an adult SAN cell.

A further characterization of this developmentally regulated newborn SAN Na⁺ channel unexpectedly revealed a pharmacological profile (i.e., high TTX and low cadmium sensitivity) that did not correspond to that of the cardiac Na⁺ channel isoform (Nav1.5) but was rather more typical of a neuronal isoform. The neuronal character of the Na⁺ channel in the newborn SAN was confirmed by in situ hybridization experiments that revealed the expression of Nav1.1 in the newborn SAN (10). This explained the functional contribution of the channel, since inactivation of neuronal Na⁺ channel isoforms typically requires greater depolarization than the cardiac isoform. Thus, I_{Na}, derived from neuronal channel isoforms would have greater availability at the typical diastolic potentials of the SAN.

The results with TTX also made it apparent that I_{Na} could not provide the only difference between newborn and adult pacemaker activity. As evident in Fig. 3, the Na⁺-independent automaticity of newborn cells unveiled by the use of TTX (33 beats/min) was significantly slower than that of adult cells (68
beats/min). Based on these results, it appears that the presence of a $I_{Na}$ during early postnatal development is a consequence of the immature state of other sinoatrial currents, which alone are not sufficient to maintain a cardiac rate compatible with metabolic requirements. This critical situation resolves during the first 40 days of life in the rabbit, thus removing the necessity for the current. It is noteworthy that while most currents contributing to pacemaker activity in the adult, such as $I_{f}, Ca^{2+}$ current, and $Na^+/Ca^{2+}$ exchanger current, are highly modulated by autonomic agonists, with cAMP-dependent processes enhancing the currents (11, 16, 119, 132), this is less true for $I_{Na}$. Modulation of $Na^+$ channels is isoform specific, and protein kinase A can be associated with either an increase or decrease in current (22, 122). For this reason, understanding of the functional contribution of $Na^+$ channel modulation to the control of pacemaker mechanisms is still unclear. However, when taken together, these observations lead to the highly speculative hypothesis that during the early postnatal period, the contribution of $I_{Na}$ ensures a failsafe mechanism protecting the animal from a potential parasympathetic/sympathetic imbalance and that the necessity for this fades during development as the autonomic nervous system reaches its mature state.

Investigation of the presence of Nav1.1 and Nav1.5 transcripts in the adult rabbit by Tellez et al. (111) found Nav1.5 in the periphery and low levels of Nav1.1 in the center, compatible with the almost negligible contribution of $I_{Na}$ to adult automaticity, as previously suggested by Baruscotti et al. (7). Interpretation of these data together with those obtained in the newborn rabbit could suggest that the expression of $Na^+$ channels in the center of the node is regulated differently than the expression in the periphery. In fact, it would be expected that Nav1.5 channels could only participate in peripheral regions due to the more negative inactivation relation of this isoform and thus are more involved in the propagation of the impulse out of the node rather than to determination of rate. These conclusions were further validated by experiments in mice having a targeted disruption of the SCN5A gene (Scn5a$^{+/−}$ mice). Peripheral SAN cells isolated from these animals displayed a reduced presence of $I_{Na}$ and reduced

Fig. 3. The contribution of $Na^+$ current to spontaneous activity decreases with postnatal development. Action potentials recorded in single cells isolated from an adult and a newborn rabbit SAN exhibited different sensitivities to the $Na^+$ channel blocker TTX (3 μM). Drug perfusion induced no significant effect (∼10.6%, $n = 5$, $P > 0.05$) on the spontaneous rate in the adult animal, whereas it profoundly (∼66.7%, $n = 5$) altered the rate in the newborn. [Modified from Ref. 7.]

Fig. 4. TTX- and nifedipine-sensitive currents elicited by ramp depolarization differ in newborn and adult SAN cells. A and B: whole cell current traces obtained in control and in the presence of TTX (3 μM) by applying a voltage ramp (from −60 to −20 mV) to single SAN cells at a depolarizing rate (35 mV/s) similar to that of spontaneous diastolic depolarization (top). TTX-sensitive current (A and B, bottom) was present in the newborn but not in the adult. C and D: similar protocols allowed the identification of nifedipine-sensitive currents (bottom) both in the newborn and adult. On average, the threshold of the nifedipine-sensitive current was 4 mV less negative in the newborn.
spontaneous rate; furthermore, experiments on intact SAN preparations confirmed that in Scn5a<sup>−/−</sup> mice, there was a significant slowing of sinoatrial conduction time and a propensity to sinoatrial block (67).

To our knowledge, thorough comparative studies of SAN current (I<sub>Na</sub>) in newborn and adult animals have not been carried out in any other species, and thus the progressive disappearance of I<sub>Na</sub> during development could be a process typical of some but not all species. Indeed, studies carried out in adult mice showed significant differences with those from rabbits. Recordings from single SAN cells revealed that the pacemaking rate in adult mice is extremely sensitive to TTX, and, in fact, I<sub>Na</sub> was recorded in almost all cells investigated (68, 75). In 2004, Lei et al. (68) concluded that although I<sub>Na</sub> is common throughout the whole node of adult mice, the molecular substrates are not the same in all regions. Cardiac Nav1.5 channels are absent from the center of the SAN but densely populate the periphery, whereas Nav1.1 channels are homogeneously distributed throughout the node. It is possible that the persistence of Nav1.1 expression in the adult mouse SAN, compared with the rabbit, reflects the need to maintain a markedly higher heart rate in the adult mouse heart. While the mechanism(s) controlling the differential regulation of Nav1.1 and Nav1.5 channels are unknown, a report (101) of developmentally regulated splice variants of the murine SCN5A gene with distinct cardiac specific enhancer regions, and the presence of homologous human splicing, suggests a direction for future research.

**Ca<sup>2+</sup> currents.** Two types of Ca<sup>2+</sup> currents are expressed in SAN cells: low voltage-activated (L-type) Ca<sup>2+</sup> channel current (I<sub>Ca,L</sub>) and high voltage-activated (T-type) Ca<sup>2+</sup> channel current (I<sub>Ca,T</sub>) (42, 117). The relative abundance of these two currents seems to be species specific since, for example, in rabbits I<sub>Ca,L</sub> is the predominant current, whereas in mice the opposite situation occurs. Although several details still need to be fully understood, there is general agreement that I<sub>Ca,L</sub> sustains the action potential upstroke (phase 0), either alone or in combination with I<sub>Na</sub>, and contributes to the later part of diastolic depolarization (76, 117, 132). The specific role of I<sub>Ca,T</sub> is more controversial since original electrophysiological studies on pacemaker cells were carried out in rabbits, a species in which I<sub>Ca,T</sub> is poorly expressed. With the more recent focus on pacemaking mechanisms in mice, studies have revealed that in this species, I<sub>Ca,T</sub> participates in diastolic depolarization (78). A further role for I<sub>Ca,T</sub> has been associated with its ability to locally trigger Ca<sup>2+</sup> release from the sarcoplasmic reticulum in atrial cells (47), although this specific action has not yet been proven in primary pacemaker cells (77).

Given their importance, the obvious question is whether Ca<sup>2+</sup> currents are subjected to developmental regulation in the SAN. As mentioned earlier, when the contribution of I<sub>Na</sub> in rabbit newborn cells is removed by TTX, the remaining automaticity is slower than that of the adult [33 vs. 68 beats/min (7)]. A logical explanation for this observation is that other ionic components crucial for nodal automaticity are still not fully mature in the newborn, and Ca<sup>2+</sup> currents are obvious candidates. Protas et al. (91) carried out a detailed investigation of developmental changes in nodal I<sub>Ca,L</sub> and I<sub>Ca,T</sub>. A comparative analysis of I<sub>Ca,T</sub> in newborn and adult rabbit SAN cells did not show any differences, thus excluding it as a major player in the developmental regulation of the SAN rate in the rabbit. Taken at face value, the absence of differences in newborn and adult I<sub>Ca,T</sub>, despite the differences in the rate in the presence of TTX, might indicate that in rabbits I<sub>Ca,T</sub> is not a critical determinant of rate. However, the only conclusion that can be definitively drawn from these data is that, to the extent that I<sub>Ca,T</sub> contributes to automaticity in the rabbit SAN, it is a developmental constant. Again, this may reflect species differences since it has been shown that the spontaneous rate of Cav3.1 (one of three T-type Ca<sup>2+</sup> channel isoforms) knockout mice is significantly slower (~10%) than that of control animals (78). In contrast, when Cav3.2 knockout mice were investigated for chronotropic performance and AV conduction of the impulse, no alterations were observed (23). Interestingly, Niwa et al. (84) showed that, in the mouse heart, Cav3.2 expression is high during embryogenesis. Opposite to Cav3.2, Cav3.1 increases during late embryogenesis and at birth to become the only expressed isoform found in the heart after birth and during adulthood (37, 84). These molecular data are consistent with the observation by Protas et al. (91) that in the rabbit, the properties of newborn and adult sinoatrial I<sub>Ca,T</sub> are not different.

More interesting are the results comparing I<sub>Ca,L</sub> in the adult and newborn (91). In particular, development acts on I<sub>Ca,L</sub> by inducing a decrease in current density (newborn: 17.6 pA/pF and adult: 12.1 pA/pF), a rightward shift of the inactivation curve (midpoint: −33.4 mV in the newborn and −28.3 mV in the adult), and a leftward shift of the activation curve (midpoint: −17.3 mV in the newborn and −22.3 mV in the adult). Recovery from inactivation was also slower in the newborn. The decreased density of the current observed in the adult would suggest a diminished contribution of this current during diastole, but the opposite is likely true when one considers that the overlapping area delimited by the activation/inactivation curves is increased in the adult due to the opposite shift of the curves. This is shown in Fig. 4, C and D, where the nifedipine-sensitive current is shown during a slow ramp clamp to mimic diastolic depolarization. While the use of nifedipine during a ramp clamp can isolate currents in addition to I<sub>Ca,L</sub>, such as a sustained inward current (I<sub>s</sub>; see Other currents and Refs. 24 and 41), it is evident that the threshold of the nifedipine-sensitive current is more negative in the adult, so that it contributes earlier in diastole. Mean data confirm this, with the measured threshold shifted 4 mV negative in the adult (n = 6–8, P < 0.05). To the extent that this reflects I<sub>Ca,L</sub>, it is thus likely that even though maximal current is higher in the newborn, the more negative activation threshold and the faster recovery from inactivation observed in the adult could explain why in the presence of TTX the rate of newborn cells is slower than that of adult cells.

The mechanisms underlying the differences observed in newborn and adult I<sub>Ca,L</sub> could arise from a developmental switch between channel isoforms. Four α-subunits of L-type Ca<sup>2+</sup> channels have been identified, and those expressed in the heart are Cav1.2 and Cav1.3, with the latter being mainly expressed in the atrium, SAN, and conduction system (12, 76, 79, 109). When Cav1.3 knockout mice were generated, SAN dysfunction and disturbances of AV conduction were observed (76, 90, 134). Analysis of sinoatrial I<sub>Ca,L</sub> in Cav1.3 knockout mice revealed significant differences with those recorded in wild type; in particular, in the absence of Cav1.3, the voltage dependence is shifted ~5 mV positive [midpoint from −16.6 to −11.4 mV (134)], and this shift demonstrates that in the...
SAN, two different isoforms of L-type $\text{Ca}^{2+}$ channels coexist. In particular, since Cav1.3 is selectively present in supraventricular regions, and since the cardiac rate is slower when this isoform is absent, it is possible that Cav1.3 in the SAN mostly contributes to diastolic depolarization, whereas contractility is contributed to by the Cav1.2 isoform. Interestingly, when the derivative of the action potential was measured at three different potentials ($-50$, $-45$, and $-40 \text{ mV}$) during diastolic depolarization in wild-type and Cav1.3-deficient mice, differences were observed only for the two more positive voltages (134). These data indicate that the contribution of Cav1.3 is indeed critical during the pacemaker phase but not in early diastole, where other currents contribute. Whether Cav1.2 and Cav1.3 are subjected to developmental regulation remains to be established. Based on the 5-mV developmental leftward shift in activation observed by Protas et al. (91) and the 4-mV shift in threshold of the nifedipine-sensitive current reported above, when considered compared with the 5-mV shift in the activation relation in Cav1.3 knockout animals (134), it could be speculated that the immature SAN expresses a prevalence of Cav1.2 channels, and postnatal development favors the expression of Cav1.3. This hypothesis also agrees with the evidence that, whereas Cav1.3 is not necessary during heart embryogenesis, the lack of Cav1.2 is lethal. Additional support for this hypothesis comes from the finding of Jones et al. (51) of a progressive loss of Cav1.2 with aging in the guinea pig SAN; however, it was not reported if a concomitant increase in Cav1.3 occurred.

Other currents. Other currents have been suggested to contribute to SAN pacemaking in the adult by various researchers, including the deactivation of the delayed rectifier $K^+$ current (48), a nifedipine-sensitive sustained cation current called $I_{\text{Na}}$ (24, 41), and $\text{Na}^+$/Ca$^{2+}$ exchange current (74). We are not aware of any studies in the immature SAN that indicate that any of these currents vary developmentally, but this is clearly a relevant question. In particular, the $\text{Na}^+$/Ca$^{2+}$ exchange mechanism merits study, as this is intimately associated with other components of Ca$^{2+}$ homeostasis such as sarcoplasmic reticular Ca$^{2+}$ stores and release mechanisms, both of which change with postnatal development in other cardiac regions (50, 57, 113).

Conclusions

We have attempted to provide a framework for considering the developmental maturation of the SAN in mammals. While any conclusions must be qualified because of the possibility of substantive differences among species, it appears the SAN undergoes substantial rearrangements both in its anatomical extension, as shown by the decrease in the Cx43 area, and in its electrical aspects, as shown by profound modifications of the ionic channel expression profile. All these aspects combine to determine an age-dependent slowing of intrinsic heart rate and conduction time. Interestingly, we observed that, whereas the contribution of $I_L$ and $I_{\text{Na}}$ to pacemaking are downregulated during postnatal life in the rabbit SAN, the opposite is true for $I_{\text{Ca,L}}$. Certainly, these events are controlled by an overall homeostatic developmental plan, but identification of the common regulators is a goal that remains for future investigations, as is extension to other species including humans. What can be stated at this time is that although some aspects are beginning to be clarified, much remains to be done before a comprehensive view of the developmental biology of SAN function will be complete.

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