Mini Review

Sino-Atrial Nodal Cells of Mammalian Hearts: Ionic Currents and Gene Expression of Pacemaker Ionic Channels

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Abstract

The cardiac pacemaker is a sino-atrial (SA) nodal cell. The signal induced by this pacemaker is distributed over the heart surface by a specialised conduction system and is clinically recorded as the ECG. The SA nodal cells are highly resistant to cardiac failure and ischemia. Under calcium overload conditions, some dysrythmias of SA nodal cells occur easily. Morphological analysis under these conditions shows swelling of the cisternae of the Golgi apparatus, with little or no other histological change or damage being observed. The rate of sinus rhythm is quite different between various species. The investigations of SA nodal cells have so far clarified the pacemaker mechanisms involved. A number of ionic channel currents or pacemaker currents, contribute to the depolarization of the pacemaker potential (phase 4). This will not occur with a single current. Recent experiments have identified several novel pacemaker currents and have also revealed several differences in the pacemaker currents between species. The marked hyperpolarization-activated inward current (If) appears in SA nodal cells of most species, while the inwardly rectifying K⁺ current (IK₁) with masked If current is found in those of the rat and monkey. In addition, the rapidly activated current (I_Kr) and slowly activated current (I_Ks) of the delayed rectifier K⁺ current (IK) contribute to the pacemaker potential in guinea pig SA nodal cells, with only the I_Ks current in porcine SA nodal cells and only the I_Kr current in the rat and rabbit. These differences in ionic channels presumably result from differences in gene expression. Some smooth muscle cells also possess the capacity to beat spontaneously. Uterine smooth muscle cells also exhibit an If current. The basal mechanism for spontaneous activity in both SA nodal cells and smooth muscle cells is almost the same, but some differences in the ionic channels and their genetic expression may contribute to their respective pacemaker currents.

Key words: Pacemaker currents, Ionic channels, Heart rate, Gene expressions, Sino-atrial nodal cells
Introduction

The sino-atrial (SA) node is known as the pacemaker region of the mammalian heart. In previous experiments using SA nodal cells, we have shown that dysrhythmias (or arrhythmias) are easily elicited under conditions involving calcium overload that occur during ischemia and cardiac failure. Clinically these SA nodal dysfunctions cause bradyarrhythmias in general and are associated with syncope but rarely with death.

In SA nodal cells, spontaneous beating or contractions is associated with interactions between ionic currents such as the L-type Ca\(^{2+}\) (\(I_{\text{CaL}}\)), the delayed rectifier K\(^+\) (\(I_{\text{K}}\)) and the hyperpolarization-activated inward (I\(_f\)) currents (Noble, 1984; Guo et al., 1995; Mitsuiye et al., 2000). Noma and colleagues (Guo et al., 1997; Shinagawa et al., 2000; Mitsuiye et al., 2000) have more recently demonstrated additional underlying currents in rat SA nodal cells, namely the rapidly activated K\(^+\) current (\(I_{\text{Kr}}\)) and the sustained inward current (I\(_s\)). These currents are major pacemaker currents in addition to the \(I_{\text{CaL}}\) and \(I_{\text{f}}\) currents. Our recent experiments have revealed some differences in the pacemaker currents found in different species. Specific differences in ionic channel distribution and their gene expression may exist, presumably resulting in differences in the sinus rate and drug sensitivity between species.

Localization and Histology

The SA node region is located on the endocardial surface at the edge of the right atrium, bounded on two sides by the superior and inferior venae cavae and around the crista terminalis between the venae cavae and the right atrial muscle. Under a lower power microscope, the SA node appears as a translucent muscular region near the sino-atrial node artery. The most prominent feature is the ring bundle, which is a thin flap of tissue that extends around most of the periphery of the node and that usually appears to be the most vigorously beating part in an isolated node.

Under the electron microscope, SA nodal cells are observed to have a relatively large nucleus and a few myofilaments (Satoh and Uchida, 1993). There are many caveolar invaginations along the surface membranes of these cells (Masson-Pevet, 1979). The intercellular space at 20 nm is wide compared with that observed in other tissues.

Isolated SA nodal cells are spindle- or spider-shaped and have a maximum length of 25–30 \(\mu\)m, with an irregular profile in cross section and a diameter of less than 8 \(\mu\)m. Isolated spontaneously beating SA nodal myocytes are curved and not flat on their base (Shinagawa et al., 2000).

Differences between Species

Determinants of sinus rate

The sinus rate of spontaneously beating SA nodal cells is said to be dependent on body weight (or size) or on the stage of pregnancy (Opthof, 2001). In isolated hearts, the sinus rate is extremely fast in small animals (\(i.e.,\) mouse, rat and rabbit), whereas it is slower in large
animals (i.e., human and elephant) (Table 1). It seems possible that the total number of spontaneous beats over the life span might be related to the metabolic rate of the animal concerned, as well as being dependent on body weight. As a result, small animals would exhibit a faster sinus rate and have a shorter life span, although this is not clear yet.

Boyett et al. (2000) have recently reported that the beating rate of cells from the rabbit SA node depends on the cell size. In addition, the phase 4 slope and $V_{\text{max}}$ are dependent on the cell capacitance (22.0 to 57.5 pF). In general, increasing the cell capacitance enhances the amplitude of the ionic channel currents, with a simultaneous increase in the sinus rate. If the cell size of an animal was related to its body weight, large animals might possess larger SA node cell sizes and thus exhibit higher sinus rates. However, this relationship is quite the opposite as larger animals possess a slower sinus rate. Also, larger animals such as the monkey and the pig do not always possess larger sized SA nodal cells. Actually, we have found no marked difference in cell size between species, although we have not examined the full range of animals in our experiments. But we conclude that there is no relationship between the SA node cell size and the body weight of different species.

**Action potentials**

Spontaneous action potentials in SA nodal cells possess several significant characteristics. When compared with the action potentials of atrial or ventricular cardiomyocytes, the SA nodal cell action potential is characterized by a pacemaker potential, seen as a slow depolarization during phase 4 diastole, which then elicits a spontaneous action potential. This is then repeated successively (Fig. 1A). In addition, a slow depolarization in phase 0 and a lack of phase 1 are observed. The maximum diastolic potential (MDP) was $\pm 50$ to $\pm 60$ mV and much lower than other cardiomyocytes, indicative of the minor contribution of the Na+ channel current ($I_{\text{Na}}$), because the $I_{\text{Na}}$ is activated at around $\pm 80$ to $\pm 90$ mV.

To generate spontaneous action potentials, it is necessary for the membrane to depolarize beyond threshold potential during the diastole of phase 4. The rate of spontaneous beating is modulated by (a) the rate of pacemaker depolarization, (b) the MDP, (c) the threshold potential, and (d) the action potential duration (APD). These factors are mostly regulated by pacemaker

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APA: action potential amplitude. MDP: maximum diastolic potential. APD50: 50% repolarization of action potential duration. CL: cycle length.
currents (which form the pacemaker potential) which flow during diastole, which is contributed to by a number of ionic channel currents, as discussed below.

**Pacemaker currents**

A number of different time-dependent currents contribute to the pacemaker activity of the SA nodal cells (Fig. 1B). In general, the regulation of the spontaneous beating SA nodal cells is considered to be due to (a) the \( I_{CaL} \) current, (b) the time-dependent decay (deactivation) of \( I_K \) conductance, and (c) the \( I_f \) current (Noble, 1984; Irisawa et al., 1993).

The \( I_{CaL} \) is the most important pacemaker current, because the generation of the pacemaker potential of SA nodal cells is almost at the same potential as the threshold potential for \( I_{CaL} \) channels. Ca\(^{2+} \) antagonists easily stop the spontaneous action potentials. At lower concentrations these antagonists suppressed and slowed just the last part of the pacemaker depolarization (Satoh and Tsuchida, 1993). A similar phenomenon was observed soon after the application of these antagonists.

The contribution of the T-type Ca\(^{2+} \) current (\( I_{CaT} \)) to pacemaking is small, and is limited to approximately the first third of the pacemaker potential (Noble, 1984), because the \( I_{CaT} \) current starts to activate at around \(-80 \text{ mV} \), which is similar to the \( I_{Na} \) channel. This is also supported by
our experiments using $I_{CaL}$ channel inhibitors (Satoh, 1995a).

The delayed rectifier K⁺ channel ($I_K$) is responsible for repolarization, and adjusts the resting potential (RP) after each action potential. The $I_K$ is comprised of three separate channels; the slowly activated ($I_{Ks}$), rapidly activated ($I_{Kr}$), and ultrarapidly activated ($I_{Kur}$) channel currents. The $I_K$ current is markedly activated during the repolarization phase (phase 3) of the action potentials, and as a result, the myocyte repolarizes back down to the RP. After repolarization during diastole, the $I_K$ slowly deactivates, which is most important for the pacemaker potential. The deactivation leads to a gradual increase in the permeability ratio of $P_{Na}/P_K$ of the membrane. This increase potentiates the pacemaker depolarization. More recently, the pacemaker mechanism of SA nodal cells has also been reported to involve $I_{Kr}$ (Guo et al., 1995; Shinagawa et al., 2000; Matsuura et al., 2002). When $I_{Kr}$ is blocked by E-4031, the spontaneous activity is depressed and sinus arrest often occurs in both rat and guinea pig SA nodal cells.

The sustained inward current ($I_s$) is also specific to SA nodal cells and is closely related to $I_{CaL}$ (Mitsuiye et al., 2000). The $I_s$ is the key pacemaker current which generates the diastolic depolarization (Guo et al., 1997; Shinagawa et al., 2000). The current is nicardipine-sensitive with a conductance of 13 pS Na⁺ current. The current density is very small (2 pA/pF) in rabbit SA nodal cells (Mitsuiye 2000). The $I_s$ is resistant to TTX (30 µM) but is highly sensitive to Ca²⁺ antagonists such as by nicardipine (0.25 to 0.5 µM) and verapamil (1 µM). Bay K 8644, a Ca²⁺ channel opener, enhances the $I_s$. Thus, $I_s$ activation makes a major contribution to the pacemaker depolarization.

The If current is “specific” to pacemaker nodal cells and is generally found in cells with spontaneous activity. It seems unlikely that this current largely contributes to pacemaking activity under normal conditions, because full activation in voltage-clamp experiments needs a greater hyperpolarization (over −70 mV) and longer duration (over 1 s) of the stimulation pulse. In the SA nodal action potentials, the MDP is approximately −60 to −70 mV and the 50% repolarization of APD (APD₅₀) is around 80 to 300 ms. Therefore, inhibition of the If current (i.e., by Cs⁺) would exhibit only a minor effect on the spontaneous activity of SA nodal cells.

The rate of pacemaker activity is strongly regulated by the autonomic nervous system. β-Adrenoceptor stimulation causes a marked positive chronotropic effect due to enhancement of $I_{CaL}$, $I_s$, and $I_{Ks}$ ionic channels. On the other hand, the opening of muscarinic K⁺ channels stimulates a substantial outward current. The hyperpolarizing shift of the MDP induced by $I_{KACH}$ activation causes a negative chronotropic effect. Simultaneously, ACh reduces $I_{CaL}$ and $I_s$.

Thus, the pacemaker potential is regulated by a complicated interaction of multiple currents under the regulation of the autonomic nervous system and is not due to a single current. Even if a particular current makes a reduced or no contribution under normal conditions, it might play a role in the generation of pacemaker depolarization and the maintenance of regular rhythm under abnormal (disease) conditions.

**Role of the Hyperpolarization-activated Inward Current (If)**

One of the most striking features of SA nodal cells is the $I_f$ (funny) current. The inward
The If current is markedly activated by a hyperpolarizing pulse. There is considerable debate over the exact role of this current. The If may increase the speed of onset of pacemaker depolarization. As mentioned above, the If current makes only a minor contribution to the pacemaker depolarization. However, cells that do not beat spontaneously do not exhibit the If. Therefore, we now consider that the If current may contribute somewhat indirectly to the diastolic depolarization involved in spontaneous beating.

The If activation depends critically on the levels of cytosolic Ca\(^{2+}\) (Hagiwara et al., 1988; Satoh, 2003). In normal cells with a cytosolic Ca\(^{2+}\) of 100 nM, the activation curve for If lies between –40 and –80 mV, suggesting that in SA node cells the If could contribute to a substantial steady inward current within the pacemaker range (–65 to –45 mV). Cs\(^{+}\) ions are relatively selective blockers of the If current. In the presence of 2 mM Cs\(^{+}\) which fully blocks the If, SA nodal cells do not cease beating spontaneously (Denyer and Brown, 1990; unpublished data). This result emphasizes that the If current is not essential for pacemaking.

In rabbit and guinea pig SA nodal cells, hyperpolarizing pulses activate marked If currents (Fig. 2A-B), whereas in rat SA nodal cells they do not always activate an If current (Fig. 2C). However, even cells without an If current can still beat spontaneously (Shinagawa et al., 2000). In mouse SA nodal cells, the functional properties of the If current resemble those of the If current in rabbit SA nodal cells (Matteo et al., 2001). In monkey SA nodal cells, as in rat SA nodal cells, the If current is not common even in spontaneously beating cells or typically beating spider-shaped cells (unpublished data). Therefore, the role of the If current seems likely to be
that of rate modulation rather than of the generation of pacemaker depolarization. But SA nodal cells that are not spontaneously beating lack an $I_f$ current. In young (3–5 day old) embryonic chick ventricular cardiomyocytes, strong spontaneous beats appear, and simultaneously a marked $I_f$ current is also identified (Satoh and Sperelakis, 1991), but both of these decrease and disappear with further embryonic development.

**Dependence on $I_{Kr}$ or $I_{Ks}$**

The delayed K+ current in SA nodal cells is comprised of both $I_{Kr}$ and $I_{Ks}$ (Sanguinetti and Jurkiewicz, 1990). The $I_{Kr}$ is rapidly activated within 200 ms while $I_{Ks}$ is slowly activated in 1 to 2 s. As shown in Fig. 3, both currents are present in rat SA nodal cells (Satoh, 1999; Shinagawa et al., 2000; Ono et al., 2000; Matsuura et al., 2002). The $I_{Kr}$ inhibition may cause a more positive MDP and a longer APD. Thus, it is likely that the sinus rate may be regulated by $I_{Kr}$ and/or $I_{Ks}$. The time-dependence of activation would be dependent on the sinus rate. The fast sinus rate found in small animals might be driven by $I_{Kr}$, whereas the slow sinus rate found in larger animals might be driven by $I_{Ks}$. The $I_{Ks}$ begins weakly at first during repolarization and then

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**Fig. 3.** Existence of $I_{Ko}$ and $I_{Ko}$ in rat SA nodal cells. A–B, Control and in the presence of E-4031. C, Activation curves for $I_{Ko}$. D, Activation curves for $I_{Ko}$. 

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gradually builds up. The rectification is delayed until the rapid phase of repolarization. The IKs channel is sensitive to β-adrenergic stimulation. On the other hand, the IKr rapidly activates to maximum intensity, then levels off. The IKr channel is insensitive to β-adrenergic stimulation, but is blocked by many antiarrhythmic drugs (e.g. disopyramide and flecainide). The IKs blockade is limited to several arrhythmic drugs such as cibenzoline and amiodarone.

As shown in Fig. 4, the spontaneous action potentials in rat SA nodal cells are suppressed by 3–5 µM E-4031 which blocks IKr. On the other hand, 293B (30 µM) which blocks IKs has a much smaller or no effect (Fig. 5). Both of these responses are observed in rat and rabbit SA nodal cells (Shinagawa et al., 2000; Lei et al., 2002) (Table 2). Therefore, the contribution of IKs to spontaneous action potentials is small under normal conditions. On the other hand, in guinea pig SA nodal cells, both IKr and IKs currents play crucial roles in the spontaneous electrical activity of these cells (Matsuura et al., 2002). In contrast, the spontaneous activity in porcine SA nodal cells depends on IKs alone and not on IKr (Ono et al., 2000). This discrepancy between different species is inexplicable at this time. Anyway, it appears from these results that spontaneous action potentials are dependent on IKr or/and IKs, but that the sinus rate is necessarily independent of these currents.

Other Pacemaker Currents

In general, there are minor pacemaker currents that contribute somewhat to the pacemaker
Cardiac pacemaker currents

Depolarization. These currents may contribute more significantly to pacemaker depolarization under some disease conditions.

The inward rectifying K⁺ (I_{K1}) channel is voltage-dependent and maintains the RP. It is well known that I_{K1} is usually absent in the SA nodal cells of rabbit and guinea pig (Fig. 2A-B), but it is present in both atrial and ventricular myocytes of these species. Actually, the I_{K1} is not present in the SA nodal cells of most species (Table 3). The lack of I_{K1} may make the membrane potential unstable after repolarization and exert a positive MDP in SA nodal cells. In rat and monkey SA nodal cells, however, a marked I_{K1} actually appeared (Fig. 2C). After application of Mg²⁺ (0.5 to 2 mM) to block the I_{K1}, a masked I_{f} current was revealed (Shinagawa et al., 2000). These results demonstrate strongly that the I_{K1} makes little or no contribution to pacemaker depolarization.

The transient outward current (I_{to}) plays an important role in action potential repolarization.

Fig. 5. Modulation by 293B of the spontaneous action potentials in rat SA nodal cells. A, Traces of the action potentials, the V_{max} and the sinus rate. B, Faster traces of the action potentials and the V_{max} as recorded at the points (a–d) indicated in panel A.
and contributes to cardiac electrical heterogeneity (Campbell et al., 1995). The expression of the \( I_{\text{to}} \) current is heterogenous (Lei et al., 2000; Decher et al., 2001; Rosati et al., 2001). The \( I_{\text{to}} \) is characterized by rapid activation and inactivation. The \( I_{\text{to}} \) has 6 segments per domain, composed of two (\( I_{\text{to}1} \) and \( I_{\text{to}2} \)) channels. \( I_{\text{to}1} \) is a voltage-dependent \( K^+ \) channel and initiates repolarization. A smaller sustained \( I_{\text{to}} \) component may also contribute to the APD (repolarization) in SA nodal cells. \( I_{\text{to}2} \) is a chloride current which is activated by \( Ca^{2+} \) (and is thus often termed \( I_{\text{ClCa}} \)).

\[ I_{\text{KACh}} \] is one of the ligand-activated channels directly activated by the acetylcholine (ACh) released from parasympathetic nerves. ACh also modulates these ionic channels due to mediation through muscarinic (M\(_2\)) receptors on the cardiac cell membranes. The SA node is precisely controlled by the autonomic nervous system with the regular rhythm largely regulated through stimulation of M\(_2\) receptors. ACh activates \( I_{\text{KACh}} \) channels during rapid repolarization and during pacemaker depolarization. Its activation lowers the MDP to a more negative potential. The gene expression of \( I_{\text{KACh}} \) is encoded by a complex containing Kir3.1 and Kir3.4 subunits (Krapivinsky et al., 1995) (Table 4). Kir3.1 and M\(_2\) receptor proteins are colocalized.

\( I_{\text{KATP}} \) channels can open under conditions of reduced cellular ATP levels (1 mM) such as

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Table 4. Gene expression of the pacemaker ionic channels in sino-atrial nodal cells.
occurs in cardiac failure and ischemia. Thus, this is a cardioprotective channel. The openers of these channels (cromakalim, pinacidil and nicorandil) caused a negative chronotropic effect that prolonged the APD in rabbit SA nodal cells (Satoh and Hashimoto, 1984; Satoh, 1993). These drugs inhibit $I_{\text{Ca}}$, but fail to affect $I_{\text{K}}$. In our laboratory, inhibition of ATP production with cyanide (5 mM), which may activate $I_{\text{KATP}}$ channels, causes a negative chronotropic effect but without the occurrence of sinus arrest. Thus, $I_{\text{KATP}}$ channels would also contribute somewhat to pacemaker depolarization.

$I_{\text{Na}}$ initiates the phase 0 depolarization of cardiomyocytes and of cardiac Purkinje fibers. However, application of TTX, a selective blocker of $I_{\text{Na}}$ channel, fails to alter the generation of spontaneous action potentials. The $I_{\text{Na}}$ may contribute to subsidiary pacemaker activity in peripheral regions, providing a backup mechanism (Warth et al., 1996; Kodama et al., 1997; Wang et al., 1998). The $I_{\text{Na}}$ has not been found consistently in SA nodal pacemaker cells. The behavior of $I_{\text{Na}}$ is somewhat similar to that of $I_{\text{Ca,T}}$ and thus contributes little to diastolic depolarization.

The background (or non-specific) inward current ($I_{\text{bg}}$) produces a gradual depolarization of the membrane during the pacemaker potential (Wilders et al., 1991; Demir et al., 1994). Sodium and chloride channel ($I_{\text{Cl}}$) currents are mostly known (Seyama, 1976; Hagiwara et al., 1992). The Na-K pump current and the Na-Ca exchanger current may play roles in the maintenance and modulation of pacemaking. The Ca$^{2+}$-activated Cl$^{-}$ current ($I_{\text{ClCa}}$), while not always present in rabbit SA nodal cells (only found in approximately one third of the cells), is activated during the pacemaker cycle (Verkerk et al., 2002). The reversal potential for Cl$^{-}$ ions of cardiomyocytes is approximately $-50$ mV (Sorota, 1999). Thus, $I_{\text{ClCa}}$ activation generates an inward depolarizing current during the pacemaker potential and generates an outward repolarizing current during phases 2 and 3 of the SA nodal action potentials.

**Cytosolic Ca$^{2+}$**

The cyclical changes in electrophysiological activity, which produce the spontaneous beats, originate as a result of the opening and closing of time- and voltage-dependent ionic channels in the sarcolemmal membrane. In addition, the establishment of this rhythm may be considered to be triggered and controlled by oscillations in the levels of cytosolic Ca$^{2+}$ due to the release of Ca$^{2+}$ from the SR (Tunwell et al., 1996). A mechanism has been found that involves T-type Ca$^{2+}$ channels triggering transient Ca$^{2+}$ release or Ca$^{2+}$ sparks (Fabiato, 1992). These Ca$^{2+}$ sparks play a prominent role in bringing the pacemaker potential to threshold in spontaneously beating atrial cells of the cat (Zhou and Lipsius, 1993; Lipsius et al., 2001). Ca$^{2+}$ release during the pacemaker potential may possibly depolarize the pacemaker potential by stimulating the inward Na$^{+}$/Ca$^{2+}$ exchange current. In our laboratory, however, switching from perforated patch-clamping to whole-cell patch-clamping (with low EGTA concentrations in the pipette solution) largely failed to affect spontaneous action potentials. Even when cytosolic Ca$^{2+}$ is not fully chelated at low concentrations of cellular EGTA (20 µM), spontaneous beats are suppressed but never abolished and the APD become relatively longer (unpublished data).

In spontaneously beating embryonic chick cardiomyocytes, the sinus rate increases with an
Increase in pCa levels (in a range from pCa 10 to pCa 6) (Satoh, 1995b). In rabbit SA nodal cells, however, ryanodine and thapsigargin have little or no effect on chronotropism. In guinea pig ventricular muscle cells, thapsigargin had a negative inotropic effect (Nario and Satoh, 1996) while in rabbit SA nodal cells, ryanodine at high concentrations caused a negative chronotropic effect (Satoh, 1997). Therefore, we conclude that in SA nodal cells, cytosolic Ca\(^{2+}\) (Ca\(^{2+}\) sparks) makes only a minor contribution to pacemaker depolarization.

**Molecular and Gene Expression**

The gene expression of the main pacemaker channels is described below, however not all ionic channels are discussed in this section.

**T- and L-type Ca\(^{2+}\) channels**

For the low voltage-activated Ca\(^{2+}\) channels (Cav), Cav3.1 and Cav3.2 encode α subunits of IC\(_{AT}\) (Cribbs et al., 1998). The gene expression of channels is summarized in Table 4. The most significant expression of the Ca\(^{2+}\) channel is Cav3.1 (α\(_{1G}\)). Cav3.1 mRNA expression is 30-fold greater in the SA nodal cells of the mouse than in the atrial cells (Bohn et al., 2000). Cav3.2 (α\(_{1H}\)) is present at a moderate level (Bohn et al., 2000). The expression of Cav3.2 is generally lower than that of Cav3.1, but it is relatively higher in SA nodal cells.

On the other hand, the predominant high-voltage activated Ca\(^{2+}\) channel, Cav1.2 (α\(_{1C}\)), encodes IC\(_{CAL}\). It is more strongly expressed in mouse SA nodal cells than in cells of the atrium (Bohn et al., 2000). The β and α\(_{2}\)δ subunits modulate the density, kinetics and activation/inactivation of the IC\(_{CAL}\) channel (Catterall, 2000). Also, a small amount of Cav1.3 (α\(_{1D}\)) mRNA is detected in mouse SA nodal myocytes. In Cav1.3-knockout mice, however, profound dysrhythmias occur (Platzer et al., 2000). Recently, a role has been established for Cav1.3 Ca\(^{2+}\) channels in the generation of spontaneous activity (Zhang et al., 2002). The half-activation voltage (V\(_{1/2}\)) is \(-17.5 ± 0.9\) mV for Cav1.3, and \(-3.9 ± 1.3\) mV for Cav1.2 (Koschak et al., 2001).

**I\(_{f}\) channel**

Despite the important physiological functions of I\(_{f}\) channels, the genes encoding these channels have only recently been identified through heterologous expression (Gauss et al., 1998; Ludwig et al., 1998; Santro et al., 1998). At least four different genes are now recognised.

Hyperpolarization-activated cation channels (HCN) contain the families of voltage-gated channels and cyclic nucleotide-gated channels (Kaupp and Seifert, 2001). These channels probably embody six transmembrane segments (S1–S6), with a pore loop between segments S5 and S6 and a cyclic nucleotide-binding domain in the C-terminal region of the polypeptide. Molecular studies have revealed that gene expression encodes HCN1 to 4 (Shi et al., 1999; Ishii et al., 1999; Moroni et al., 2000; 2001; Moosmang et al., 2001). The HCN transcripts are mostly present in SA nodal cells, and are 25 times more abundant in those cells than in Purkinje fiber cells (Shi et al., 1999). The four different mammalian genes involved display high homology. HCN1 is abundantly expressed in the brain, but also in the SA node. HCN2 expression is detected in both the brain and the heart, but is at very low levels in the SA node. HCN3 mRNA
is detected in the brain, but at lower levels than the other three subtypes (HCN1, 2 and 4). HCN4 mRNA is detected at high levels in both the brain and the heart. Strong HCN4 expression has also been detected in the rabbit SA node. In addition, moderate amounts of HCN4 mRNA have also been detected in both rat neonatal and adult ventricles and also in human tissue (Ludwig et al., 1999; Shi et al., 1999; Ishii et al., 1999; Moroni et al., 2001).

**I\textsubscript{k} channels**

There are two types of I\textsubscript{k} channels, namely the I\textsubscript{Kr} and I\textsubscript{Ks} channels. The I\textsubscript{Kr} channel is composed of two proteins, the \( \alpha \) subunit being ERG and the \( \beta \) subunit being MiRP1. The ERG protein transcript is closely correlated with the presence of I\textsubscript{Kr} in both ferret and rabbit SA nodal cells (Brahmajothi et al., 1997; Wymore et al., 1997). The I\textsubscript{Ks} channel is composed of two different proteins, the \( \alpha \) subunit being KvLQT1 and the \( \beta \) subunit being minK. MinK transcripts are more abundant in the SA node than in either the atrium or ventricle (Brahmajothi et al., 1996). It has been reported that a minK-related protein, MiRP1, enhances the density and activation rate of I\textsubscript{f} channels (Yu et al., 2001). MiRP1 mRNA is highly expressed in rabbit SA nodal cells, which suggests that it makes a possible contribution to the pacemaking functions of SA nodal cells. The details of the I\textsubscript{k} channels are summarized in Table 4.

The I\textsubscript{K1} channel has only two transmembrane segments in each domain. Kir2.1 is expressed as the predominant cardiac I\textsubscript{K1} subunit (Brahmajothi et al., 1996). The I\textsubscript{KAC} channel is formed by complexes containing Kir3.1 and Kir3.4 subunits (Krapivinsky et al., 1995). The protein Kir3.1 is present in SA nodal cells of rat, ferret and guinea pig, while the protein Kir3.4 is found in rat SA nodal cells (Dobrzynski et al., 2001). Cardiac channel expression of I\textsubscript{KATP} channels is encoded as Kir6.2 and SUR2A (Tucker et al., 1995; Chutkow et al., 1996; Babenko et al., 1998).

**I\textsubscript{f} channel**

Gap-junctional hemichannel connexin (Cx) proteins are the basis of intercellular electrical coupling (Beyer et al., 1990). The SA nodal cells are shielded against hyperpolarizing atrial influences by compartmentalization of Cx expression (Coppen et al., 1999). In the SA nodal cells of both rabbit and human hearts, Cx45 and Cx40 are both expressed, but Cx43 is absent (Ninomiya, 1966; Beyer et al., 1990). Cx40 alone is expressed in 55% of canine SA nodal cells. Cells expressing all 3 types of connexin are located in bundles abutting atrial tissue, whereas Cx40 expressing cells are located in central SA nodal cells (Kwong et al., 1998). Thus, myocytes coexpressing Cx40, Cx43 and Cx45 are found from the SA nodal cells into the atrium, and would transmit pacemaker impulses to drive the atrium.

**Myocardial Contraction**

Spontaneous beats of the heart are produced as a result of repeated myocardial contractions of SA nodal cells, due to generation of spontaneous action potentials regulated by the underlying ionic channels. Sympathetic stimulation of Ca\textsuperscript{2+} channels increases the force of myocardial contraction. Simultaneously, the spontaneous beat is stimulated by the enhanced Ca\textsuperscript{2+} channels (CICR) involved with the SR (both in the exterior cell membrane and in the T tubules).
Sympathetic stimulation increases the number of Ca\(^{2+}\) ions (mostly of CICR) available for binding to actin TnC sites, resulting in enhancement of the force of myocardial contraction.

Once Ca\(^{2+}\) ions are released by actin-myosin dissociation, the excess Ca\(^{2+}\) ions must be quickly removed from the cytosol. Sympathetic stimulation of dormant SRCa\(^{2+}\) ATPase pumps (by G protein phosphorylation) effectively accelerates the transport of Ca\(^{2+}\) from the cytosol into the SR. Phospholamban, a membrane peptide adjacent to some SR Ca\(^{2+}\) ATPase pumps, acts as a brake to inactivate them. The phosphorylation of phospholamban brings about its inactivation of the SR pump; in effect, sympathetic stimulation activates these dormant SR Ca\(^{2+}\) ATPase pumps. Thus, as a result, the excess Ca\(^{2+}\) is removed from the cytosol.

**Future Directions, and the Automaticity of Smooth Muscle Cells**

The pacemaker mechanisms of SA nodal cells from mammalian hearts can now be understood. Pacemaker activity of the SA nodal cells is a pendulum movement between depolarization and repolarization. The pacemaker depolarization is the initial stages of depolarization followed by phase 0 of the depolarization. These events are summarized in Figure 6. Visual spontaneous beats are the result of repeated contractions. While we are in the process of investigating and elucidating the mechanisms involved in monkey SA nodal cells, it is still unclear whether the same mechanisms are involved in human SA nodal. Further extensive studies are needed to elucidate in more detail the as yet unknown mechanisms involved in spontaneous activity of SA nodal cells (including the molecular and genetic expression of pacemaker ionic channels). It is expected that such a study will be effective and beneficial for
the clinical treatment of pacemaker dysfunctions such as sick sinus syndrome.

Finally, in smooth muscle cells, the foundation of the pacemaker mechanism is very similar to that found in SA nodal cells. It is again a pendulum movement with a repetitive depolarization and repolarization. In spontaneously beating rat pregnant uterus smooth muscle cells, an I_f current is identified although not markedly (Satoh, 1995c). It has also been shown that the spontaneous activity of smooth muscle cells may be largely dependent on transient Ca^{2+} sparks similar to atrial pacemaker cells. The Ca^{2+} sparks are able to activate Ca^{2+}-dependent K^+ channels (I_{Ca,K}) to produce the repolarization between spontaneous action potentials (Nelson et al., 1995) as well as Ca^{2+}-activated Cl^- channels (I_{Ca,Cl}) to produce the depolarization during pacemaker potential (ZhuGe et al., 1998). The I_{Ca,K} current in aortic smooth muscle cells is inhibited by β-adrenoceptor and muscarinic receptor stimulation and also by PK-C stimulation (Satoh, 1996). Interstitial cells of Cajal, the gastrointestinal pacemakers, exhibit Ca^{2+} release from IP_3-dependent Ca^{2+} stores (IP_3,R) activating a Ca^{2+}-dependent cationic current that drives pacemaker depolarization (Thomsen et al., 1998). The Ca^{2+} release is sensitive to a variety of second messenger signaling mechanisms in the cytosol. These mechanisms are a potential site for the regulation of spontaneous activity by autonomic neurotransmitters, hormones or drugs.

References


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