Mini Review

Pacemaker Mechanism of Porcine Sino-atrial Node Cells

Kyoichi ONO1, Shigehiro SHIBATA1 and Toshihiko IJIMA1

1Department of Pharmacology, Akita University School of Medicine, Hondoh 1-1-1, Akita 010-8543, Japan

Abstract

In cardiac sino-atrial node (SAN) cells, time- and voltage-dependent changes in the gating of various ionic currents provide spontaneous, stable and repetitive firing of action potentials. To address the ionic nature of the species-dependent heart rate, action potentials and membrane currents were recorded in single cells dissociated from the porcine SAN, and compared with those from SAN cells of rabbits, guinea-pigs and mice. The porcine SAN cells exhibited spontaneous activity with a frequency of 60–80 min⁻¹, which was much slower than that of rabbit SAN cells. Under voltage clamp conditions, depolarization activated the L-type Ca²⁺ current (I_{CaL}) followed by a gradual activation of the delayed rectifier K⁺ current (I_{K}) while hyperpolarization activated the hyperpolarization-activated cation current (I_{h}). It was found that the major component of I_{K} in porcine SAN is the slowly activating I_{K} (I_{Ks}), in contrast to SAN cells of the rabbit and other species in which the rapid I_{K} (I_{Kr}) plays an active role in repolarization and the subsequent pacemaker depolarization. Replacement of rabbit I_{Kr} with porcine I_{Ks} and a slight modification in the gating parameters and amplitudes of other current systems in the ‘Kyoto Model’ gave an adequate reconstruction of spontaneous action potentials as well as of the voltage clamp recordings. We conclude that the density and the kinetics of I_{K} contribute, in part, to the different heart rates of various species.

Key words: slowly activating I_{K}, pacemaker activity, simulation

Introduction

The spontaneous activity of the sino-atrial node (SAN) determines the heart rate in various mammalian species. When single cells were dissociated from the SAN region, they still exhibited spontaneous activity in physiological saline solution. However, when the cells are voltage-clamped at a given membrane potential using a patch electrode, neither contraction nor oscillatory change of the membrane current is observed, except in pathological conditions such as Ca²⁺ overload. This indicates that, in cardiac pacemaker cells, the spontaneous action potential and resulting contraction is brought about through time- and voltage-dependent changes in the gating of ionic channels (Irisawa et al., 1993), though a different mechanism has also been

Correspondence to: K. Ono, Department of Pharmacology, Akita University School of Medicine, Hondoh 1-1-1, Akita 010-8543, Japan
Phone: +81-18-884-6066 Fax: +81-18-836-2603 e-mail: onok@med.akita-u.ac.jp
reported in cat atrial pacemaker cells (Huser et al., 2000). The alternative activation of inward and outward current systems produces a feedback loop which generates the oscillation of the membrane potential. A large number of ionic currents in single SAN cells have been identified (Irusawa et al., 1993), and, using the conductance and gating parameters of the individual current components, it is possible to reproduce the pacemaker activity by computer simulation (Yanagihara et al., 1980; Noble and Noble, 1984; Wilders et al., 1991; Demir et al., 1994; Dokos et al., 1996; Zhang et al., 2000; Kurata et al., 2002). Figure 1 shows an example of the pacemaker model simulation, in which the action potential and ionic currents in rabbit SAN cells were reproduced using the Kyoto Model developed by Sarai et al. (2003). In this model, 13 ionic currents have been incorporated. The currents include L-type Ca\(^{2+}\) current (I\(_{\text{CaL}}\)), T-type Ca\(^{2+}\) current (I\(_{\text{CaT}}\)), the delayed rectifier K\(^+\) current (I\(_{\text{K}}\)), Na\(^{+}\)-K\(^+\) pump current (I\(_{\text{NaK}}\)), muscarinic K\(^+\) current (I\(_{\text{KAChe}}\)), hyperpolarization-activated cation current (I\(_{\text{h}}\)), background cation current and a dipyridamole-sensitive sustained inward current (I\(_{\text{NaCa}}\)), Na\(^{+}\)-Ca\(^{2+}\) exchange current, I\(_{\text{NaCa}}\), Na\(^{+}\)-K\(^+\) pump current.

**Fig. 1.** Spontaneous action potentials and underlying ionic currents in a rabbit SAN cell. The action potentials and ionic currents were drawn by using the Kyoto Model. The arrows indicate the zero current level. The cell capacitance is 32 pF. I\(_{\text{CaL}}\), L-type Ca\(^{2+}\) current; I\(_{\text{CaT}}\), T-type Ca\(^{2+}\) current; I\(_{\text{KAChe}}\), muscarinic K\(^+\) current; I\(_{\text{NaK}}\), Na\(^+\)-K\(^+\) pump current; I\(_{\text{NaCa}}\), Na\(^+\)-Ca\(^{2+}\) exchange current; I\(_{\text{NaCL}}\), Na\(^+\)-K\(^+\) pump current.
During the later part of the pacemaker potential, several inward currents such as $I_h$, $I_{CaT}$ and $I_{st}$ participate in driving the membrane towards the threshold of the following action potential. It should be noted, however, that most studies were carried out using rabbit hearts, and the simulated action potentials only represent the automaticity of the rabbit heart, which has a heart rate of approximately 200 min$^{-1}$. To get insight into the pacemaker mechanism of the hearts of other species including the human heart, we have attempted to isolate the pacemaker cells of the porcine SAN. The porcine heart rate, ranging between 60–80 min$^{-1}$ in the resting condition, is much slower than that of the rabbit (Shibata et al., 1999; Ono et al., 2000). On the basis of the action potentials and the membrane currents recorded in porcine SAN cells, the ionic mechanisms underlying the species difference in heart rate is discussed.

**Morphology and Action Potential**

Typical pacemaker cells of the porcine SAN are morphologically similar to those of other species (Irisawa et al., 1993; Ono et al., 2000; Ono and Ito, 1995; Cho et al., 2003). They were visually identified as long, spindle shaped cells in control Tyrode’s solution. They showed faint striations and prominent, centrally located nuclei.

Figure 2 shows typical records of spontaneous activity obtained by the nystatin-perforated patch-clamp technique in porcine, rabbit and murine SAN cells. The overall electrical activity of SAN cells is qualitatively similar between the various species. Quantitatively, however, the action potential of the porcine SAN cells was characterized by a much slower beating rate, pacemaker depolarization and upstroke of the action potential, and by a longer duration of the action potentials.
potential, when compared to those of the rabbit and murine preparations. The beating rate was approximately 82 min⁻¹, 202 min⁻¹ and 410 min⁻¹ in pig, rabbit and murine SAN cells respectively. The parameters of the action potentials are summarized in Table 1. It is clear that the SAN cell beating rate reflects the heart rate of each species. It was also noted that the maximum upstroke velocity was slowest and the duration of the action potential was longest in porcine SAN cells.

### Whole-cell Current of Porcine SAN Cells

Voltage clamp studies revealed qualitatively similar current systems in SAN cells of various species. One of the characteristics of SAN cells may be the existence of $I_h$ (DeFrancesco et al., 1083; Irisawa et al., 1993). With hyperpolarizing voltage clamp pulses, the initial jump of the current is very small, indicating that there is little or no inward rectifier $K^+$ current in SAN cells (Fig. 2, lower panels). Instead, a slowly activating inward current due to $I_h$ was observed in SAN cells of all species. $I_h$ starts to activate around −70 mV, and the half-activation was observed at −90 − −100 mV (Shibata et al., 1999). The current was blocked completely by 2 mM Cs⁺.

Upon depolarization a transient inward current due to the L-type Ca²⁺ current was followed by a slowly activating delayed rectifier K⁺ current ($I_{Ks}$). The cardiac $I_K$ consists of two components, the rapidly ($I_{Kr}$) and the slowly activating $I_K$ ($I_{Ks}$) (Sanguinetti and Jurkiewicz, 1990). It has been reported that $I_K$ is largely due to $I_{Ks}$ in rabbit SAN cells (Ito and Ono, 1995; Ono and Ito, 1995; Verheijck et al., 1995). In porcine SAN cells, on the other hand, it appears that $I_{Ks}$ plays a major role in repolarization and the subsequent pacemaker depolarization.

### The Delayed Rectifier K⁺ Current in Porcine SAN Cells

$I_{Ks}$ is characterized as a slowly-activating $I_{Ks}$, and the activation threshold is around −20 mV and $V_{1/2}$ is about +10 mV. The time course of activation and deactivation requires several seconds and the fully-activated current-voltage relationship shows little rectification. By contrast, $I_{Ks}$ is activated at a more negative potential ($V_{1/2}$ is around −25 mV) and shows strong inward-rectification (Sanguinetti and Jurkiewicz, 1990; Ito and Ono, 1995). These currents can also be distinguished pharmacologically. $I_{Ks}$ is specifically blocked by the class III

### Table 1  Action potential parameters of isolated SAN cells

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pig</th>
<th>Rabbit</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beating rate (min⁻¹)</td>
<td>80.5</td>
<td>203.9</td>
<td>294</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>75.1</td>
<td>95.8</td>
<td>79.4</td>
</tr>
<tr>
<td>$V_{max}$ (V s⁻¹)</td>
<td>1.37</td>
<td>10.3</td>
<td>14.5</td>
</tr>
<tr>
<td>APD₀ (ms)</td>
<td>196.6</td>
<td>NA</td>
<td>42</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>−58.4</td>
<td>−70.7</td>
<td>−56.7</td>
</tr>
<tr>
<td>DDR (mV s⁻¹)</td>
<td>34.4</td>
<td>68.7</td>
<td>NA</td>
</tr>
</tbody>
</table>

$V_{max}$: maximum upstroke velocity; APD₀, action potential duration; MDP, maximum diastolic potential; DDR, diastolic depolarization rate. NA: not analyzed.
antiarrhythmic agent E4031 (Sanguinetti and Jurkiewicz, 1990), whereas \( I_{\text{Ks}} \) is inhibited by chromanol 293B (Busch et al., 1996; Fujisawa et al., 2000). Ono et al. (2000) has shown that the \( I_k \) of porcine SAN cells possesses electrophysiological and pharmacological properties quite similar to those of \( I_{\text{Ks}} \). The reversal potential of \(-75 \text{ mV}\), measured with 5.4 mM external and 150 mM internal \( K^+ \), is \(-10 \text{ mV}\) positive to the equilibrium potential expected for a \( K^+ \)-selective electrode from the Nernst equation. E4031 had no effect on porcine \( I_K \) (Fig. 3A), but chromanol 293B completely blocked it (Fig. 3B). This is in contrast to the finding that in rabbit SAN cells,
the E4031-sensitive IKr was predominant (Ono and Ito, 1995; Ito and Ono, 1995; Verheijck et al., 1995). The porcine IKs was activated at potentials more positive than that of the rabbit IKr (Fig. 3C). Furthermore, blockage of IKs inhibited spontaneous action potentials of porcine SAN cells. All these findings indicate, not only that IKs is the dominant outward K+ current, but also that IKs plays a functional role in the pacemaker activity of porcine SAN cells.

It should be noted that the properties of IKs appear to be different, depending on the mammalian species. The half-activation voltage was +32 mV in guinea-pig SAN cells (Anumonwo et al., 1992) and +20 mV in porcine SAN cells (Ono et al., 2000). The relationship between the time constants of activation and the membrane potentials showed a clear bell-shaped curve peaking near 0 to +20 mV in guinea-pig SAN cells (Anumonwo et al., 1992). By contrast, voltage dependence of porcine IKs was not obvious at potentials more positive than +10 mV (Ono et al., 2000). Furthermore, the values for fast and slow time constants are generally slower in porcine IKs (Ono et al., 2000) than in the IKs of guinea-pig SAN cells (Anumonwo et al., 1992). These properties might be beneficial for forming the longer action potential duration in porcine SAN cells, compared to that in guinea-pig SAN cells.

It has been reported that IKr exists in SAN cells of a small species such as the rabbit (Ito and Ono, 1995; Ono and Ito, 1995; Verheijck et al., 1995), guinea-pig (Anumonwo et al., 1992; Guo et al., 1997) and mouse (Cho et al., 2003), and that it plays an important role in repolarization and the resulting slow diastolic depolarization. Although IKr was not recorded in porcine SAN cells that showed little evidence of striations, it did exist in large, rod-shaped quiescent cells in the same preparations (Ono and Iijima, unpublished data). IKs and IKr have been reported to distribute heterogeneously in the SAN region (Lei et al., 2001; Boyett et al., 2003). Molecular studies have revealed that the minK channel (KCNE1), which is considered to form IKs channels along with another K+ channel subunit, KvLQT1 (KCNQ1) (Barhanin et al., 1996; Sanguinetti et al., 1996), is expressed in the SAN region of the guinea-pig (Freeman and Kass, 1993), ferret (Brahmajothi et al., 1996) and mouse (Kupershmidt et al., 1999). On the other hand, the mRNA of ERG, which forms IKr (Lei et al., 2001) together with another subunit, MiRP1 (KCNE2) (Abbott et al., 1999), is expressed substantially in the SAN region of the rabbit heart (Wymore et al., 1997) and in the crista terminalis of the ferret heart including the SAN region (Brahmajothi et al., 1997). All these findings indicate that the distribution of IKs and IKr varies not only with the mammalian species but also with the SAN region. In this respect, it is of interest to know the distribution and function of IKr and IKs within the SAN region of pigs.

Other Current Systems

As noted above, Ih, a peculiar ionic current in SAN cells, shows essentially similar biophysical properties in the various species examined (DiFrancesco et al., 1983; Shibata et al., 1999). Also, no significant difference in the Ih current density is observed between porcine and rabbit SAN cells (Fig. 2). Moreover, blocking Ih using 2 mM Cs+ reduced the spontaneous activity of porcine SAN cells only by 10%. These findings indicate that, although Ih exists in SAN cells and may play a role in pacemaker activity (DiFrancesco et al., 1983), Ih by itself cannot account for the difference in the heart rate of various species.
Pacemaker mechanism of porcine SAN cells

Ist may be another unique current system in SAN cells (Lei et al., 2001; Matsuura et al., 2002; Cho et al., 2003). Ist is activated at –60 mV with little inactivation. The current is carried by Na\(^{+}\), and blocked by organic and inorganic Ca\(^{2+}\) antagonists. Although the molecular nature of Ist has not been clarified, an essentially similar current has been recorded in porcine SAN cells (Ono, unpublished data). As for other current systems, however, little is known whether the gating properties or the current density of these currents differ between rabbits and pigs.

Computer Simulation of Porcine SAN Activity

Since various current systems in SAN cells seem to share common properties in different species, it might be possible to reproduce the spontaneous activity of porcine SAN cells using the Kyoto Model (Sarai et al., 2003). We modified several parameters in the model to simulate porcine action potentials. Firstly, the amplitude of I\(_{Kr}\) was reduced to almost zero and the density of I\(_{Ks}\) was incorporated. Secondly, a slight retardation of I\(_{CaL}\) inactivation was necessary to reproduce the plateau phase of porcine AP. This seemed to be appropriate because the voltage clamp analysis revealed that the sustained component of I\(_{CaL}\) was much larger in porcine SAN cells than in those of rabbits (Ono, unpublished data). In fact, the steady-state current-voltage relationship, measured in control Tyrode’s solution, showed an N-shaped curve with a negative slope of around –20 mV (Ono et al., 2000). Finally, the amplitude of the background currents was adjusted so as to simulate the whole-cell current in the voltage-clamp mode.

Figure 4 illustrates the result of simulation. For comparison, the original model for rabbit SAN cells is also shown. The overall properties of the action potential are quite similar to those of those that have been recorded (Ono et al., 2000). The simulated porcine SAN cell displays repetitive action potentials with a frequency of 64.6 min\(^{-1}\). The maximum diastolic potential is

![Fig. 4. Delayed rectifier K\(^{+}\) currents (I\(_{Ks}\), I\(_{Kr}\)) and I\(_{CaL}\) during spontaneous pacemaking in rabbit and porcine SAN models, formulated by the Kyoto Model.](image-url)
–51 mV and the maximum upstroke velocity is 2.6 Vs$^{-1}$. In both SAN cells, the rising phase of the action potential is due to $I_{CaL}$ and the repolarization is caused by the inactivation of $I_{CaL}$ and the activation of $I_{K}$. Because of the kinetic difference between rabbit $I_{Kr}$ and porcine $I_{Ks}$, the plateau of the action potential is prolonged and the rate of repolarization is slower in porcine SAN cells than that of rabbit SAN cells. In other words, the relatively longer duration of the action potentials in porcine SAN cells enables $I_{Ks}$ to be activated substantially, thereby contributing to the repolarization. This idea is supported by the finding that, although a substantial amplitude of $I_{Ks}$ was recorded in guinea-pig SAN cells under voltage clamp conditions, $I_{Ks}$ makes only a slight contribution to repolarization, but $I_{Kr}$ plays a major role (Anumonwo et al., 1992; Guo et al., 1995; Matsuura et al., 2002). The slow kinetics of $I_{Ks}$ may also help to induce the following gradual change of the membrane potential in porcine SAN cells. Thus, the difference in the beating rate of SAN cells is explained, at least in part, by the difference in the kinetics of $I_{K}$ between the two species.

It should be emphasized that not only $I_{K}$ but also a variety of other ionic currents are important in forming an action potential. To simulate the relatively longer duration of the action potential, it was necessary to slow the inactivation of $I_{CaL}$ in the present study. Furthermore, the amplitude of $I_{CaL}$ was model-adjusted to mimic the slow uptake of the action potential. These were done only for practical purpose. Detailed analysis of $I_{CaL}$ and other individual current are clearly necessary to elucidate the pacemaker mechanism of porcine SAN cells. Nevertheless, the present results and simulation may provide guidance for future analysis of pacemaker activity in a large variety of mammalian species including human beings.

**Conclusion**

This study shows that $I_{Ks}$ plays an essential role in regulating action potential duration and pacemaker activity in porcine SAN cells. This is in contrast to the fact that in rabbit and guinea-pig SAN cells, $I_{Kr}$ plays a major role. The large variation in the frequency of SAN cell activity between different species might be accomplished, at least in part, by the differences in the gating properties and the amplitude of $I_{Kr}$ and $I_{Ks}$.

**Acknowledgments**

This work was supported by grants from the Ministry of Education, Science Sports and Culture of Japan.

**References**


(Received September 5, 2003; Accepted September 16, 2003)