Modelling Study on Acetylcholine Regulation to the Pacemaker Ability of the Sinoatrial Tissue in Hearts

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Abstract. As an important neurotransmitter, acetylcholine (Ach) is closely related with dysfunction of sinoatrial node (SAN), but many questions about its effects on electrophysiological behaviors of SAN are still unclear. In this paper based on the dynamic model of rabbit SAN and atrial cells while considering Ach activated inward rectifying K’ current, other Ach-adjusted ionic currents, and heterogeneity of SAN as well, a two-dimensional tissue model was developed. Computer simulation studies found that slowing of the firing rates caused by Ach could reach above 300%. Upstroke velocity of the central cell is over 3 times greater than that of the peripheral cell, thus presenting more sensitivity to Ach and easier property of sinus pause. Additionally, when Ach distributed nonuniformly, the leading pacemaker site would shift to where the maximum concentration gradient of Ach was. Moreover, the reentrant wave produced at atrial tachycardia could invade into the SAN and suppress its spontaneous firing. The greater the Ach concentration, the easier the suppression is.

1. Introduction

As a pacemaker site, the sinoatrial node (SAN) in hearts contains abundant nerve fibers, so its automatic depolarization is undoubtedly regulated by vagal nerve activity. Researches have illustrated that it is the neurotransmitter released from vagal nerves that acts on dynamic activities of the ionic channel through membrane receptors and consequently effecting electrophysiological behaviors of cardiac myocytes, so as an important neurotransmitter, acetylcholine (ACh) is sure to participate in determination of the automaticity of SAN. In addition, atrium can also act on electrical activities of SAN by loading effects. Optical mapping experiment has observed the close relationship between dysfunction of SAN and atrial arrhythmia\(^{[1,2]}\).

So far there have been some reports about relationship between electrophysiological function of SAN and Ach. For example, Moss\(^{[3]}\) studied the important effect of muscarinic receptor (M2R) on the SAN by developing a model to describe the relation of Ach concentration and M2R. Karpaev\(^{[4]}\) studied the role of fibroblast-myocyte coupling in the SAN activity with a Ach-adjusted model. Zhang\(^{[5]}\) studied effects of released Ach on SAN automaticity in sodium channel gene mutation models. These researches have improved our knowledge of Ach in SAN, however, because of the small size and thin wall as well as heterogeneous structure of SAN, the function of Ach to the cells at different sites and the effects of nonuniform distribution of Ach and the ectopic beats are still unclear. The present paper quantitatively studied these questions by introducing Ach-regulated equations into the developed SAN...
and atrium tissue model.

2. Methods

2.1. Development of the tissue model

Three ionic currents have been established to be regulated by ACh under basal conditions, among which Ach-activated inward rectifying K⁺ current, $I_{K,Ach}$, plays a key role. So equation (1) was introduced to the central and peripheral of the rabbit SAN model,

$$I_{K,Ach} = g_{K,Ach} / k \times \frac{[Ach]^{1.5}}{(2.8 \times 10^{-7})^{1.5} + [Ach]^{1.5}} \times \frac{[K^+]_o}{10 + [K^+]_o} \times \frac{V - E_K}{1 + e^{(V - E_K - 140)F/2.5RT}}$$  \hspace{1cm} (1)$$

where $g_{K,Ach}$ is the maximum conductance, $j$ and $k$ are inactivation variables, $[Ach]$ is the Ach concentration, $[K^+]_o$ is the extracellular K⁺ concentration, $V$ is the membrane potential, and $E_K$ is the K⁺ equilibrium potential.

The L-type Ca²⁺ current is reported to be partially inhibited by Ach, so its maximum conductance, $g_{Cal,max}$, was fractionally blocked by equation (2).

$$g_{CaL} = g_{Cal,max} \times (1 - 0.56 \frac{[Ach]}{0.9 \times 10^{-4} + [Ach]})$$  \hspace{1cm} (2)$$

In addition, Ach is reported to suppress the hyperpolarization-activated current, $I_h$, by shifting its activation curve to more negative potential, so as given by equation (3), the shift $s$ in the membrane voltage was also introduced.

$$s = -7.2 \times \frac{[Ach]^{0.85}}{(1.25 \times 10^{-8})^{0.85} - [Ach]^{0.85}}$$  \hspace{1cm} (3)$$

SAN coupling with an atrium tissue was then developed by the excitation-diffusion equation. To address regional differences between central and peripheral SANCs, conductance of gap junction, capacitance and size of the sinoatrial cell were changed via the exponential scaling factor. Since SAN in an adult rabbit is reported to be about 6 mm × 2 mm in the superior to inferior vena cava direction, the developed SAN tissue model in present paper was set to be a slice of 60 × 20 cells with 0.1 mm space step.

2.2. Numerical solution and tachycardial induction

Excitation-diffusion equation was solved by the operator splitting method in which the ordinary differential equation for every single cell and the partial differential equation for the electrical diffusion were solved separately by Euler and five-point difference schemes.

The cross field technique was used to induce electrical reentry in the study in which a plane wave from left to right was initiated by applying stimulation of 2 ms duration and 30 μA/μF amplitude at the left-hand side 10 × 70 cells. When a gradient in refractoriness was established, another stimulus was delivered over the lower 70 × 10 cells, the wave front then curved and finally formed the spiral wave and reentry.

3. Results and discussion

3.1. Effects of Ach on different sites

In present paper the effects of Ach activation on action potential and rhythmic firing are analyzed. Figure 1 shows action potentials and their change rates in cycle length (CL) for central (a and b) and peripheral (c and d) cells. The dotted, dashed and solid lines correspond to Ach concentrations of 0.0
M, $3 \times 10^{-8}$ M and $6 \times 10^{-8}$ M in (a) and 0.0 M, $16 \times 10^{-8}$ M and $25 \times 10^{-8}$ M in (b), respectively.

As shown in (a) and (c), no matter to the central or peripheral cells, Ach activation can prolong CL and reduce amplitude of action potential with different extent. Although these alterations are present in both cell types, changes for the central cell are obviously greater than that for the periphery, especially at low Ach concentration. Pacing pause of the center appears when Ach concentration increases to $8 \times 10^{-8}$ M. In contrast, alteration of the peripheral cell is not remarkable at low Ach concentration. However, when Ach is over $15 \times 10^{-8}$ M, CLs of the periphery increase quickly by $35\%$ and even $319\%$ at concentrations of $16 \times 10^{-8}$ M and $30 \times 10^{-8}$ M. The automaticity is lost over $33 \times 10^{-8}$ M. Thereby, it can be seen that the central cell is more sensitive to the Ach activation and easily loses its rhythmic firing than the periphery. As observed in animal experiments\cite{11}, the cell with slower upstroke velocity (UV) of the action potential is more sensitive to Ach and vagus nerve stimulation. UV of the dashed action potentials in (a) and (c) are measured to be 2.79 mV/ms and 9.98 mV/ms, respectively. Obviously, the central cell has much smaller UV than its periphery, thus presenting more sensitive characteristics to the nervous stimulation.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Action potentials and change rates in CL for different Ach concentrations}
\end{figure}

\subsection*{3.2. Electrical diffusion regulated by Ach}
Figure 2 displays electrical conduction and action potentials along the recording line in the developed tissue model at uniform distribution of Ach. The Ach concentration set in Figure 2 is $3 \times 10^{-8}$ M and the start time is considered the time when the left-hand central cell begins to fire. As shown in (a), the rhythmic firing is produced and starts to propagate to the right at about 30 ms. At 130 ms in (b) the peripheral cells have been depolarized with excitation diffusion to the atrium. When the atrium is gradually excited as shown in (c), the whole tissue begins to repolarize as (d) and finally returns to the resting potential. The above electrical propagation and pacing rhythm can also be seen from the action potentials on the recording line in (e). For the other Ach concentration, similar activations can be found. So although the peripheral cell loses its automaticity at higher Ach, however, because of the gap junction and load effect of the atrium, the leading pacemaker site still locates at the center when Ach is uniformly distributed.

Meanwhile, it is found that for the nonuniform distribution of Ach the shift of leading pacemaker site takes place. Figure 3 gives action potential and its conduction at inhomogeneous Ach distribution.
of $6 \times 10^{-8}$ M. In (a) and (b), Ach distributes from the center to the left-hand end of 3 mm and 4.5 mm, respectively. As shown by "*" marks, spontaneous excitations are generated first at the boundary of Ach taking effect, thus producing the electrical propagation towards the central SAN and atrium separately and making the site with the maximum gradient of Ach concentration the primary pacemaker. A lot of researches$^{[2,6]}$ implicate an important role of heterogeneous setting for automaticity of SAN. As indicated by the three arrows in Figure 1(a), we can find that with increased release of Ach, the maximum diastolic potential (MDP) becomes more negative, thus imposing a greater hyperpolarizing effect, and consequently, a delay of diastolic depolarization. So it makes the cells without Ach get a chance to fire first, and finally resulting in a shift of the primary pacemaker site.

Moreover, the effect of atrial tachycardia on SAN automaticity under the condition of Ach activation is studied. Figure 4 shows electrical behaviors at Ach concentration of $3 \times 10^{-8}$ M. As noticed in (a) and (d), if the whole potential in SAN is more negative when the front of reentrant wave arrives at the SAN-atrial border, the SAN cells can be depolarized and generate a conduction from peripheral to central SAN along the direction of the solid arrow. Then because of the high-speed rotation, when the reentry arrives at the SAN-atrial border once again, the peripheral cells are still in the refractory period, thus blocking the wave from entering into the SAN as denoted by "*" marks in (b) and (d). At the third time of arrival, due to the short action potential duration and short repolarization time of the peripheral cells, the reentrant wave is able to form a partial retrograde conduction along the direction of the broken arrows in (c) and (d). After that, a similar cycle is repeated until the end of reentry. Obviously, automaticity of the SAN is completely inhibited during the above process. In addition, it is found that with elevation of Ach concentration, beating rhythm of SAN becomes slow, thus making the time at resting state prolonged and easily leading to a retrograde excitation and suppression of the spontaneous firing.

Animal experiment$^{[1]}$ found that SAN was characterized as the lowest frequency region surrounded by the higher frequency atrial region, suggesting that the increment ectopic in atrium can suppress
automaticity of SAN. Findings in this paper not only are in good agreement with the reported phenomenon, but also can display a complete process of retrograde excitation from the high frequency atrial region into the SAN. Since a long time is required for the SAN repolarization, as shown in Figure 4 a Wenckebach-like conduction of 4:1 is formed in which for every 4 excitations only one can be observed to enter the SAN and produce a complete conduction, and that is the cause of the low frequency SAN region.

4. Conclusion
In this paper effects of Ach release on SAN pacemaker ability are quantitatively analyzed by developing a Ach-regulated SAN and atrial mathematical model. It is found that activation of Ach can make the beat slow by 150% for the central cell and even 300% for its periphery. At the low Ach concentration the change ratio of beat frequency for the center is much greater. Meanwhile, the central cell presents easier property to lose rhythmic firing, thus exhibiting more sensitivity to Ach. When distribution of Ach is nonuniform, the primary pacemaker site shifts to the location of the maximum concentration gradient of Ach. Moreover, at the occurrence of atrial tachycardia, the reentrant wave can conduct into the SAN and suppress its automaticity. The higher the Ach concentration, the easier the inhibition is. The above findings not only illustrate a close relationship between nervous regulation and electrical behaviors of SAN, but more importantly make Ach regulation in automatic rhythm, pacemaker site and effects of ectopic much clear. It is helpful for further understanding the relation and intrinsic mechanism of nervous system and arrhythmia.

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References
[1] Fedorov, V.V., Chang, R., Glukhov, A.V. (2010) Complex interactions between the sinoatrial node and atrium during reentrant arrhythmias in the canine heart. Circ., 122: 782–789.
[2] Oren, R.V., Clancy, C.E. (2010) Determinants of heterogeneity, excitation and conduction in the sinoatrial node: a model study. PLoS Comput. Biol., 6:e1001041.
[3] Moss, R., Sachse, F.B., Moreno-Galindo, E.G., et al. (2018) Modeling effects of voltage dependent properties of the cardiac muscarinic receptor on human sinus node function. PLoS Comput. Biol., 14: e1006438.
[4] Karpaev, A.A., Syunyaev, R.A., Aliiev, R.R. (2018) Effects of fibroblast-myocyte coupling on the sinoatrial node activity: a computational study. Int. J. Numer. Meth. Biomed. Eng., 34: e2966
[5] Zhang, J.Q., Li, X., Liang, L.S., et al. (2013) Effects of external stimuli on the pacemaker function of the sinoatrial node in sodium channel gene mutations models. China Life Sci., 56: 818-822.

Figure 4. Effects of reentry in atrium on electrical activities of the sinoatrial node at 3×10⁻⁸ M Ach
[6] Zhang, H.G., Holden, A.V., Noble, D., et al. (2002) Analysis of the chronotropic effect of acetylcholine on sinoatrial node cells. Cardiovasc. Electrophysio., 13:465-474.

[7] Majumder, R., Jangsangthong, W., Feola, I. (2016) A mathematical model of neonatal rat atrial monolayers with constitutively active acetylcholine-mediated K+ current. PLoS Comput. Biol., 12: e1004946.

[8] Garny, A., Kohl, P., Hunter, P.J., et al. (2003) One-dimensional rabbit sinoatrial node models: benefits and limitations. J. Cardiovasc. Electrophysio., 14:121-132.

[9] Zhang, H., Holden, A.V., Kodama, I., et al. (2000) Mathematical models of action potentials in the periphery and center of the rabbit sinoatrial node. Am. J. Physiol. Heart Circ. Physiol., 279: 397-421.

[10] Li, X., Zhang, J.Q., Shuai, J.W. (2014) Isoprenaline: a potential contributor in sick sinus syndrome-insights from a mathematical model of the rabbit sinoatrial node, Scientific World J., 2014: e540496.

[11] Vinogradova, T.M., Fedorov, V.V., Yuzyuk, T.N., et al. (1998) Local cholinergic suppression of pacemaker activity in the rabbit sinoatrial node. J. Cardiovasc. Pharmacol., 32: 413-424.