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Cardiac electrophysiology: Past, present and future

Part II

Membrane currents in cardiac pacemaker tissue

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Summary. The present work is a brief survey of the mechanism of the cardiac pacemaker in sinoatrial node cells. Information on the pacemaker mechanism in cardiac tissue has been greatly enhanced by the development of the single cell isolation technique and the patch clamp technique. These methods circumvent to a large extent the difficulties involved in voltage clamping multicellular preparations. The calcium current (I_{Ca}), delayed rectifier potassium current (I_K), transient outward current (I_{to}; I_A), and the hyperpolarization activated inward current (I_h or I_f) were found both in whole cell preparations and in single channel analysis. The physiological significance of these currents, together with the exchange current systems for the pacemaker depolarization are discussed.

Key words. Pacemaker cell; ionic currents; patch clamp; single channel current; sinoatrial node cells; single cell.

Introduction

At the time when I started to work on membrane currents in Prof. Silvio Weidmann's laboratory (Hallerianum, Bern) in September, 1969, many people believed that it was impossible to control the membrane potential of the mammalian pacemaker cells. No information on the membrane currents of the pacemaker cells was available at that time, and thus the pacemaking mechanism of the nodal cell could only be assumed from the voltage clamp data derived from Purkinje fibers and squid axons. The early attempts at voltage clamping of the sinus node, such as the double sucrose gap method[24], or the small isolated preparations of nodal tissue[46, 47], possessed all the limitations inherent in multicellular preparations. The introduction of single cells for the study of nodal currents greatly simplified the geometry and offered favorable conditions for whole cell voltage clamping. Single cells possess the advantage that the extracellular space of the multicellular preparations has been eliminated. However, it must also be borne in mind that accumulation/depletion can still occur in the caveolae or the T system of these cells. Use of a patch clamp pipette allows internal dialysis of the cells and the effects of ATP and various intracellular ion concentration changes on different membrane current systems can be investigated[27, 34, 44, 47, 56].

While internal dialysis allows separations of current systems, buffering of $[Ca]_i$ by the introduction of EGTA can abolish Ca currents normally present if EGTA is not added[15, 16].

The nodal region contains several different cell types which can be separated on a morphological basis and by electrophysical criteria[37–40]. Despite these differences, they all appear to show qualitatively similar action potentials and currents after isolation. However, in reports on isolated cells sufficient detail should be given so that cells can be identified (e.g. cell size, capacity).

Resting potential of nodal cells

In pacemaker cells the equivalent of the resting potential is the potential at which the net current flow is zero under voltage clamp conditions. In the S-A node this lies between -30 and -40 mV. Initially this low resting potential of the nodal cells was attributed to the high permeability of the membrane for sodium ions and a low intracellular potassium concentration[56]. However, as shown in figure 2, the potassium reversal potential in 2.7 mM $[K]_o$ was estimated to be -100 mV, indicating that E_K is approximately the same as that found in ventricular cells[47]. This finding is in good agreement with the potassium activity measurements of Grant and Strass[20], who found an a_K^i of 85 ± 3 mM at a $[K]_o$ of 4 mM. Moreover, the slope conductance in nodal cells did not change significantly when sodium was replaced by tris chloride[50], suggesting that a large P_{Na} is not the major factor for the low resting potential. Since the inward rectifier K channel is almost absent in the nodal cell, the cause of the high P_{Na}/P_K of the pacemaker cell is most likely due to a low potassium conductance.

Results on whole cell voltage clamps from four different laboratories agree that the leakage and time-independent current components in the nodal cell are small in the physiological voltage range investigated[14, 19, 21, 38–40, 50].

At the resting potential the slope resistance of the nodal cells was between 12 and 15 K Ω cm^2, while that of the ventricular cell was less than 1 K Ω cm^2 [50]. The high membrane resistance in the nodal cell means that a small change in the membrane current could cause large changes in the membrane potential and be important for the generation of the pacemaker potential.

Ionic currents underlying the pacemaker potential

Three time-dependent current systems underlie the pacemaker potential, namely a) potassium current, b) calcium current and c) the hyperpolarization-activated inward current[5, 26].

These three systems will now be considered separately.

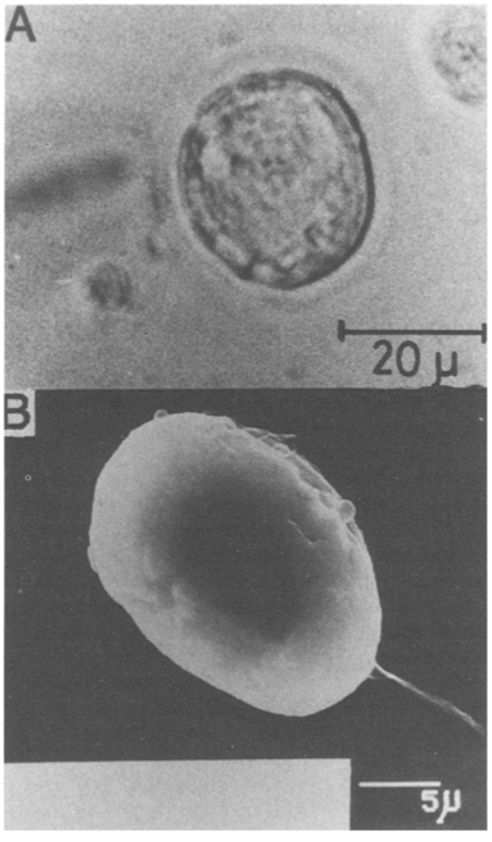

Figure 1. Single S-A nodal cell: *A* photomicrograph of an isolated S-A node cell in 1.8 mM Ca Tyrode solution. *B* Scanning electronmicrograph of an isolated S-A node cell. (From Nakayama et al.[38])

a) *Potassium current.* The traditional view is that it is the deactivation of the outward K current that is at least partially responsible for the pacemaker depolarization[5, 19, 43]. Recently, Shibasaki and Irisawa[54] have proposed that the kinetics of the K current inward rectifying may be more complicated than those found by Yanagihara and Irisawa[58]. Since the delayed K channel seems to have fast inactivation kinetics as well as slow activation kinetics it seemed necessary to reconsider the K current kinetics in the nodal cell. Shibasaki gave a series of equations to express K current, based on the experimental findings, which resemble those described recently by Noble and Noble[44] on a purely theoretical basis. There are several ligand gated K channels in the myocardium. The ATP-sensitive K channel[28, 45], the acetylcholine-activated K channel[53, 55], the sodium-activated K channel[29], etc. It would be interesting to know how these different K channels contribute to the pacemaker current, but there is at present no detailed information for the S-A node.

b) *Calcium current.* From experiments with multicellular preparations it was suggested that not only deactivation of the K current but also activation of the slow inward current (I_{si}) plays a significant role in the pacemaker depolarization[26, 58, 59]. For instance Noma, Kotake and Irisawa[48] found that adrenaline in such preparations increased not only the slope of the depolarization but also the slow inward current. However, our proposal that I_{si} plays a major role in the pacemaker depolarization did not gain general acceptance because in multicellular preparations the activation threshold for I_{si} was difficult to determine exactly.

It has recently been found that I_{si} contains at least two components, a calcium current component (I_{ca}) and a slower current component sensitive to EGTA within the cell. In the whole cell clamp mode I_{ca} can be isolated when the patch pipette contains Cs and EGTA. Threshold measurements for the activation of I_{ca} gave values similar to those found in multicellular preparations. However, in this method the EGTA-sensitive slow component is greatly reduced[15, 16].

The most clear-cut demonstration of I_{ca} is obtained with single channel analysis from nodal cells where Ca currents are not much different from those obtained in ventricular cells[9, 21, 23]. Two kinds of Ca channels have also been found in atrium[2], ventricle[36, 41], embryonic heart[52] and in neural cells[1, 8, 10, 51]. The transient type of Ca channel (I_T) is activated at more negative potentials than the slowly inactivated Ca channel (I_L). The inactivation of I_T is rapid at the plateau potential level but relatively slow in the pacemaker potential range. Moreover, the density of I_T is larger in S-A nodal cells than in atrial or ventricular cells[21]. If Ni (40 μM) which specifically blocks I_T is added to S-A nodal cells the depolarization phase is slowed. Nilius[42] has modified the model of Yanagihara et al. and found that repetitive activity could be stopped if I_T was not included in the model. We conclude from all this evidence that the transient type of Ca current participates significantly in the generation of the pacemaker potential.

c) *Hyperpolarization-activated inward current.* Inward current activated by hyperpolarization is called I_h, I_f, or I_p[3, 4, 35, 57] and this current plays an important role in the acceleration of the heart rate in response to adrenaline[3, 48]. I_h is not directly related to the nodal cell rhythm, as the absence of I_h was often observed in the isolated nodal cells or in spontaneously beating multicellular A–V nodes. The kinetic properties of the I_h indicate that the time course of activation of this current is of the order of 1–2 s and is thus too slow for influencing the frequency of the primary pacemaker. Cs, which is known to block I_h, does not greatly affect the pacemaking properties of nodal cells[49].

We thus concluded that the role of I_h for the pacemaker depolarization within the normal cardiac cycle range is minor. Maylie and Morad[35] and DiFrancesco et al.[14], on the other hand, consider that the I_h plays a significant role for the pacemaker activity. Brown et al.[7] have recently shown a single channel current of the order of less than 0.1 pA in the

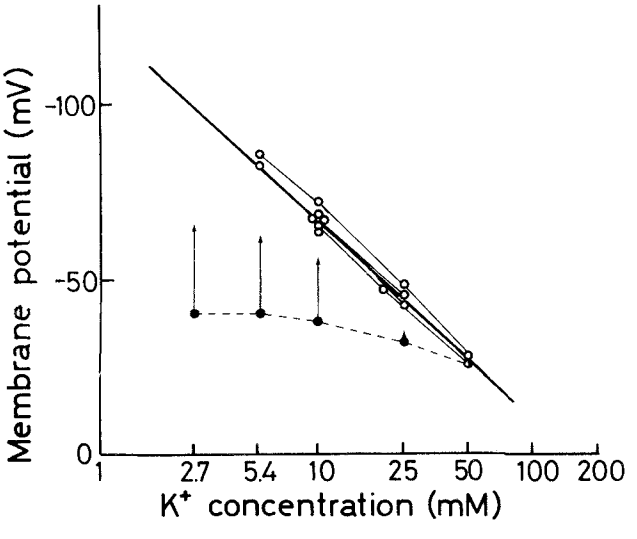

Figure 2. Resting potential of S-A node cells. Resting potential (closed circles) at various $[K]_o$. Point of arrow indicates the maximum diastolic potential. Open circles are the reversal potentials of the pacemaker current components, obtained in 4 different experiments. Thick line was fitted by the least squares method and its slope was 58 mV for a ten-fold increase in $[K]_o$. (From Noma and Irisawa[47])

Synoptic presentation of the different single K channel conductances

Potassium channel	Single channel conductance (pico Siemens) a	b	Voltage dependency	Presence in node	Physiological significance
Delayed rectifier	1.6	10	+	+	Pacemaker depolarizatic repolarization
Inward rectifier (formerly named I_{K_1})	6.0	46	+	–	Resting potential plateau phase
K (ATP)	35	80	–	+	Action potential duratic
K (ACh) (confused with I_{K_1})	13	66	+	+	Vagal effect
K (Na)		207	–	?	Action potential duratic
K (Ca)		100	–	?	Early repolarization
K (T_o, A) (I_{To} = abbreviation of transient outward current, or A current in neural cell)			+	+	Short duration

Single channel conductance: *a* at physiological K concentration (5.4 mM); *b* at symmetric K concentration.

S-A node cells. They found that the apparent number of channels varied greatly from cell to cell, which suggests that I_h channels may not be uniformly distributed on the membrane surface.

Whether I_h contributes to the pacemaker potential in the normal cardiac cycle range or not is still unsettled. In several examples we failed to observe this current in A–V nodal cells, even though they were rhythmically active[32, 38]. Because of this we consider that I_h is not essential for the beat-to-beat change in pacemaker potential. Although the hyperpolarization-activated current is often referred to as 'the pacemaker current', the role of this current is most probably to maintain a low resting membrane potential to protect the cell from a prolonged hyperpolarization. In this sense the current contributes to the setting of the cardiac rhythm.

d) *Transient outward current.* Transient outward current is known to exist in many pacemaking cells[12, 22]. It can help to shorten the action potential duration and it ultimately modulates the frequency of the rhythm. It is interesting that the transient outward current has been recorded in pacemaking cells from the crista terminalis, A–V node, and S-A node[18, 21, 39]. However, contributions of this current system to the slow diastolic depolarization have not yet been shown (fig. 3).

Recently, in sheep Purkinje fiber, this current was found to have a calcium-dependent activation (Carmeliet; personal communication). Its exact role for the pacemaker is at present obscure. From the review given so far it can be concluded that there exists no single current system which can elicit the normal pacemaker potential. Progressive decay of K current and the T type Ca current might attribute to the slow diastolic depolarization, whereas I_h possibly contributes to modulating the rhythm by changing the resting membrane potential.

e) *Inward background current.* To elicit the slow diastolic depolarization a net inward current is a necessary prerequisite[59]. I_h, as mentioned above, can act as a kind of a 'background current', since it has very slow kinetics compared to the heart rate. The Na-K pump current could also be a candidate for the background current, but the direction of this current is most likely to be outward (fig. 4). The current-voltage relations of the Na-K pump current reveal that the pump outward current was activated at about −150 mV and almost saturated at potentials positive to −50 mV[17]. Thus is seems unlikely that the Na-K pump current is important in the pacemaker potential range; the accumulation and the depletion of K^+ at the cleft may elicit pump current oscil-

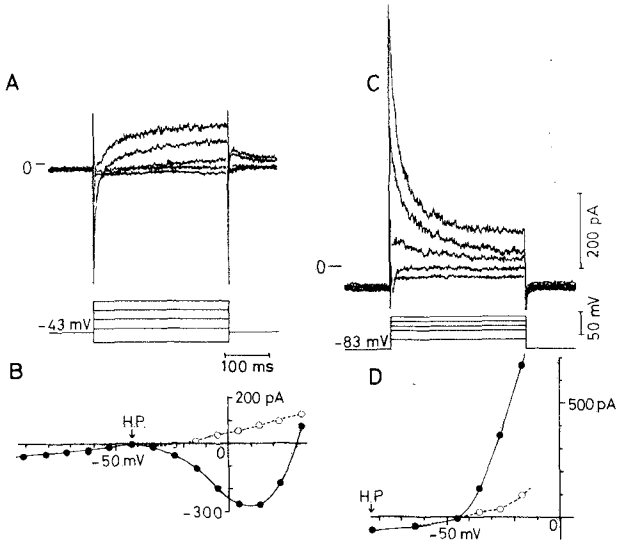

Figure 3. Transient outward current in A-V node cells. *A* Holding potential −41.0 mV; pulses applied with 20 mV steps. *C* When the potential was held at −80 mV, the depolarizing pulse to 0 mV elicited a transient outward current. The current voltage relationships are given below (*B* and *D*). (From Nakayama and Irisawa[39])

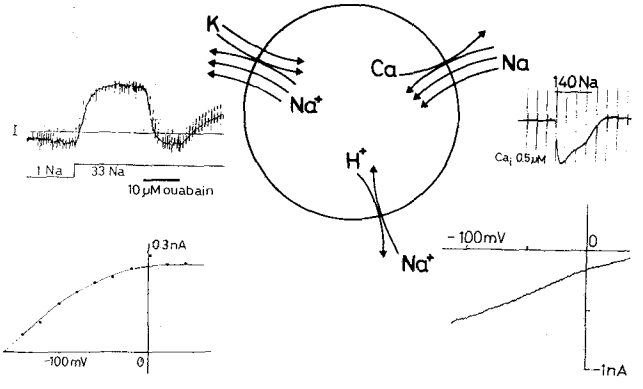

Figure 4. Ionic currents carried by pump and exchange current systems. Upper left: The outward current was elicited when 33 mM Na^+ was loaded in the cell. The outward current was abolished under the effect of 10 μM ouabain. Vertical lines superimposed on the current trace are the currents in response to repeated test pulses. Lower left: Current voltage relation of the Na-K pump current, isolated from a guinea pig ventricular cell. At potentials positive to 0 mV, the pump current was saturated. Assuming this relation fits to the nodal cell, pump current at the range of pacemaker depolarization is outward. Upper right: Inward Na-Ca exchange current was elicited when the intracellular Ca concentration was kept at 0.5 μM and the extracellular Na concentration was increased from 0 to 140 mM. Lower right: The current voltage relationship of the exchange current indicates that the Na-Ca exchange current is inward near the pacemaker potential range. The stoichiometry of the Na-H exchange system is neutral and no current is detectable. (By courtesy of J. Kimura)

lations, but in the physiological range of $[K]_o$ the pump current is likely to be maintained at an outward current level. Na-Ca exchange could also act as an inward background current. Recently, Kimura et al.[31] found an inward Na-Ca exchange current under the condition of 10 mM Na_i, 0.2 μM Ca_i, 140 mM Na_o and 1 mM Ca_o at potentials negative to −10 mV. As the membrane potential becomes more negative, the inward current tends to increase (fig. 9 in Kimura et al.[31]). Since Danielson's measurement[13] on $[Na]_i$ gives a value of $[Na]_i$ of 16.7 mM, the above conditions may not be far from the normal physiological state (fig. 4).

It is well known that when $[Ca]_i$ is increased under the influence of cardiotonic steroids, ventricular cells exhibit a transient depolarization after a train of driven action potentials or a transient inward current after a depolarizing clamp pulse[30,33] in voltage clamp experiments. This trasient inward current increase is attributed to the Na-Ca exchange current and/or opening of a non-specific cation channel of the type described by Colquhoun et al.[11]. The transient inward current has been observed in Purkinje fibers and ventricular cells[30,33]. In the small S-A node specimen, the small oscillations in current and potential were also found[25]. Brown et al.[6] found that this oscillatory current in the nodal cell could be attributable to the transient inward current and that a large fraction of this current is generated by Na-Ca exchange. Only a relatively small fraction of these oscillatory currents may be generated by the non-specific current. The nonspecific cation channel has a reversal potential at 0 mV and at potentials negative to this reversal potential, the current is inward. Thus if intracellular Ca within the pacemaker cell increases to a level which activates this inward current, it could generate a part of the inward background current. There is, however, still no experimental support for the presence of the nonspecific cation channel in the nodal cell.

Conclusion

Using single S-A node cells as a simplified model of the cardiac pacemaker, the action potential and the membrane currents were studied. The action potential of the pacemaker cell has a slow diastolic depolarization phase which is followed by a relatively rapid rising phase of depolarization. After the overshoot, the membrane potential repolarizes to the most negative phase. Ionic currents responsible for the pacemaker depolarization were investigated with the patch clamp method. Two major ionic currents, i.e. potassium current and calcium current, are responsible for the pacemaker depolarization. The hyperpolarization-activated inward current helps in modulating the heart rate by regulation of the resting membrane potential. The Na-Ca exchange current might play a significant role for the inward background current.

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Contribution of the Na^+/K^+-pump to the membrane potential

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Summary. The inward movement of sodium ions and the outward movement of potassium ions are passive and the reverse movements against the electrochemical gradients require the activity of a metabolism-driven Na^+/K^+-pump. The activity of the Na^+/K^+-pump influences the membrane potential directly and indirectly. Thus, the maintenance of a normal electrical function requires that the Na^+/K^+-pump maintain normal ionic concentrations within the cell. The activity of the Na^+/K^+-pump also influences the membrane potential directly by generating an outward sodium current that is larger when the Na^+/K^+-pump activity is greater. The activity of the Na^+/K^+-pump is regulated by several factors including the intracellular sodium concentration and the neuromediators norepinephrine and acetylcholine. The inhibition of the Na^+/K^+-pump can lead indirectly to the development of inward currents that may cause repetitive activity. Therefore, the Na^+/K^+-pump modifies the membrane potential in different ways both under normal and abnormal conditions and influences in an essential way many cardiac functions, including automaticity, conduction and contraction.
Key words. Active transport of ions; cardiac tissues; electroneutral and electrogenic Na^+/K^+ pump; control of Na^+/K^+-pump; normal and abnormal electrical events.

The 'necessity' of a sodium-potassium pump

The concentration of sodium is far greater outside than inside the cardiac cells whereas the opposite verifies for potassium ions. In addition, the inside of the cells is negative at rest. This creates an inwardly directed electrochemical gradient for the sodium ions. At rest, the sodium conductance is relatively small and so is the background sodium current. However, the large sodium gradient is exploited in several ways. Because of the inwardly directed electrochemical gradient, it is only necessary to open the fast sodium channels to initiate a fast inward current through the cell membrane