



Mechanisms Underlying Cardiac Pacemaker Activity

Mario Vassalle*

*Department of Physiology and Pharmacology, State University of New York,
Downstate Medical Center, Brooklyn, New York, USA*

Generally, the pacemaker activity is equated to diastolic depolarization. However, there is no agreement about the mechanisms underlying the pacemaker potential either in the sino-atrial node (SAN) or in Purkinje fibers. In fact, even within the SAN, there are dominant and subsidiary pacemakers and this raises the question as to whether there are two (dominant and subsidiary) pacemaker mechanisms and whether each of them can sustain spontaneous discharge. Also, both mechanisms appear to be present in subsidiary cells depending on the degree of polarization. In addition, it is becoming apparent that diastolic depolarization is only part of the mechanism leading to spontaneous discharge. This is not too surprising since DD is an afterpotential (it follows an action potential) and therefore it could not be responsible for the initiation of pacemaker activity after a period of standstill. In fact, both in SAN and in Purkinje fibers there are oscillatory pre-potentials (ThV_{os}) that are responsible for the initiation of spontaneous discharge. These same oscillatory potentials are also needed for the maintenance of spontaneous activity, since they are the obligatory link between diastolic depolarization and the threshold for the upstroke. Another oscillatory after-potential (V_{os}) appears involved in spontaneous activity. V_{os} appear to contribute to the faster discharge of the sino-atrial node under normal conditions and to that of Purkinje fibers under abnormal conditions. The aim of this review is to evaluate the different mechanisms that have been proposed for diastolic depolarization in the sino-atrial node and in Purkinje fibers and illustrate the findings that show the importance of voltage oscillations in the initiation and maintenance of pacemaker discharge in SAN and in Purkinje fibers.

Key words: cardiac pacemaker activity, diastolic depolarization, diastolic oscillatory potentials, sino-atrial node, Purkinje fibers

INTRODUCTION

The necessity of a diastole and a systole in the cardiac cycle requires that cardiac cells should be activated rhythmically. For cardiac fibers to do so, the resting potential has to decrease by the end of each diastole to the threshold. The threshold could be defined as the potential at which a net inward current initiates a regenerative depolarization and therefore the action potential (AP). In turn, the AP is the electrical signal leading to contraction. The process of the attainment of the threshold in the different cells of the heart must be a sequential one in order to develop an organized cardiac contraction. Thus, the threshold must be attained first in a discrete site (normally

the sino-atrial node) and from there the action potentials should propagate in an orderly fashion to atria and ventricles.

This obligatory sequence illustrates the need for two different mechanisms to attain the threshold: *diastolic depolarization* in dominant pacemakers in the sino-atrial node (SAN) and *conduction* of impulses in the rest of the cells of the heart. Thus, the SAN (and only the SAN) sets the pace at which all other cells of the heart will be activated according to the patterns of conduction. The location of the pacemaker of the heart in the SAN also facilitates the control of the rate of discharge. Thus, the SAN is heavily supplied with sympathetic and parasympathetic fibers and it is sufficient to modify the rate of the SAN through release of autonomic neuromediators to change the rate of the heart.

If, outside the SAN, the threshold is attained in whatever fashion outside of conduction of impulses originating from the SAN, an arrhythmia (such as extrasystoles) will occur. An extreme example of an abnormal attainment of the threshold is fibrillation, the major disturbance of which is the attainment of the threshold in different cells as soon as they again become excitable. This results in total disorder in cardiac excitation and contraction.

Typically, safety factors are present also for the pace-

Received: May 15, 2003; Revised: July 9, 2003; Accepted: July 11, 2003.

*Corresponding author: Mario Vassalle, Dept. of Physiology, S.U.N.Y., Downstate Medical Center, Box 31, 450 Clarkson Ave., Brooklyn, NY 11203, USA. Tel: +1-718-270-1158; Fax: +1-718-270-3103; e-mail: mario.vassalle@downstate.edu

Communicated by Cheng-I Lin, Department of Physiology & Biophysics, National Defense Medical Center

maker activity of the heart. Subsidiary pacemakers are needed that can insure rhythmic discharge of the heart and prevent cardiac standstill if SAN dominant pacemakers or conduction of impulses originating from SAN dominant pacemakers fails. Such subsidiary pacemakers are present within the SAN, in the atria, atrio-ventricular node and Purkinje fibers. A widespread location of subsidiary pacemakers is required by the fact that initiation of spontaneous discharge by subsidiary atrial pacemakers or atrio-ventricular node would be of little use if an atrio-ventricular block is present.

If subsidiary pacemakers become spontaneously active, their diastolic depolarization (DD) too must attain the threshold for the upstroke by the end of diastole. This creates a potential conflict with the activity of SAN and it is a possible source of arrhythmia. This conflict is generally avoided by the fact that DD of all subsidiary pacemaker is less steep than that of SAN dominant pacemakers. This means that, under normal conditions, depolarization by the process of conduction will bring the membrane potential of subsidiary pacemakers to the threshold before the local DD has a chance to do so.

In addition to that, another powerful mechanism is present, namely, overdrive suppression^{1,2}, which maintains DD of subsidiary pacemakers more negative (and therefore away from the threshold) than it would be in the absence of this factor. This suppression of spontaneous discharge of subsidiary pacemakers is frequency-dependent. This is rather convenient in that the suppression of their discharge is maintained as long as subsidiary pacemakers are overdriven by SAN (and therefore are not needed as actual pacemakers) and it is removed when the subsidiary pacemaker discharge is needed (e.g., SAN standstill or complete atrio-ventricular block).

Thus, the initiation of heart beat is strategically located in a site (SAN) near the *venae cavae* so that the activation of atria can precede that of ventricles and the process of conduction insures that the activation of cardiac cells is sequential (and not simultaneous or disorderly). While under normal conditions the heart is continuously and rhythmically active, under abnormal conditions either impulse formation or conduction to ventricles can fail (e.g., disease or strong vagal stimulation). This raises substantial questions about both the initiation and maintenance of spontaneous discharge.

We shall see that DD is necessary but not sufficient for spontaneous discharge and that the link between DD and threshold is provided by voltage oscillations. Whether or not a drug treatment in a patient is suitable might depend on our understanding of what is involved in spontaneous

discharge of different pacemakers (e.g., lidocaine in complete heart block)³. The significance of this problem stems from the fact that the initiation and maintenance of pacemaker discharge are of paramount importance for survival.

We shall review the evidence that indicates that diastolic depolarization is caused by the different pacemaker currents in different pacemaker tissues and that diastolic oscillatory potentials ThV_{os} are (and V_{os} may be) an obligatory link between diastolic depolarization and the threshold in determining pacemaker activity.

DIASTOLIC DEPOLARIZATION AND PACEMAKER CURRENTS

As the very name indicates, this depolarization occurs in diastole and therefore after the action potential (AP). This raises the question as to the relationship between action potential and DD. If a pacemaker tissue is quiescent (e.g., when it is superfused in high $[K^+]_o$), an electrical stimulus can evoke an AP which is followed by an undershoot to the maximum diastolic potential (MDP) and a subsequent return to the resting potential through DD. This behavior shows that DD: (i) is an afterpotential and that (ii) is not necessarily associated with spontaneous discharge.

The undershoot to the MDP is caused by the fact that during an AP a net outward current develops that is responsible for that subsequent hyperpolarization. The hyperpolarization to the MDP can not be maintained because the net outward current decays in the diastolic potential range as a function of time, thereby causing diastolic depolarization. One possibility is that during the depolarization associated with the AP either an outward current carried by K^+ ions is activated (e.g., the delayed rectifier current I_K) or an inward current is deactivated (e.g., the hyperpolarization-activated I_p). A calcium current is ruled out, because it is activated during the AP and its subsequent decay during diastole would cause not an undershoot but a diastolic repolarization toward the resting potential.

However, the current underlying DD does not need to be the same in dominant and subsidiary pacemakers within and without the SAN, since the resting potential and DD range are more negative in subsidiary pacemakers. It needs to be added that not every current present during diastole qualifies as a pacemaker current. The pacemaker current is the *conditio sine qua non* for the presence of diastolic depolarization. It is the time- and voltage-dependence of this current that permits the potential to decline during diastole toward the threshold. Other currents may modify DD, but that does not make any of them a "pacemaker current". However (as mentioned above), we shall see that

DD is not the only factor that is needed for spontaneous discharge.

Sino-Atrial Node

1. Dominant and subsidiary pacemaker mechanisms

Because DD is an afterpotential, the underlying pacemaker current could be related to the type of AP that precedes it. Hence, the necessity to consider if different APs are followed by different pacemaker mechanisms, also in view of the fact that the pacemaker site may shift within the SAN under different conditions. In fact, the SAN is not a homogenous structure from the electrical and morphological points of view, since it includes dominant and subsidiary pacemakers^{4,5}. SAN dominant APs are slow responses with a MDP of -50/-60 mV. Instead, SAN subsidiary pacemakers have a fast upstroke and a more negative (~-80 mV) maximum diastolic potential^{4,6}.

Several currents have been proposed as *the* pacemaker current of SAN: the delayed rectifier current I_K ; the hyperpolarization-activated inward current I_p ; the slow inward current I_{Ca} ; the steady state current and even time-independent inward currents⁷⁻⁹. Which of these currents might be the pacemaker current of SAN subsidiary pacemakers is generally not taken into consideration.

In SAN dominant cells (when quiescent), the resting potential of -30/-40 mV⁷ is too negative for the activation of I_K and too positive for the activation of I_f . I_K activates on depolarization from the resting potential and deactivates slowly on repolarization⁷. This creates an outward tail which indeed would cause an undershoot and subsequent DD¹⁰.

Instead, I_f would not deactivate during an AP elicited electrically from the resting potential of -30/-40 mV, because it is not activated at the resting potential and therefore it can not deactivate during the AP, thereby causing the undershoot to the MDP and subsequent DD. Thus, I_f activates slowly only on hyperpolarization from a holding potential of about -50 mV^{7,10}.

The situation is different for subsidiary pacemakers within the SAN for two major reasons. Their diastolic range is negative enough to allow the activation of I_f on repolarization to the MDP and it is negative to the reversal potential for I_K (-65 mV¹¹). Therefore, it is likely that I_f is the SAN subsidiary pacemaker current¹⁰, since in the subsidiary diastolic range the decay of I_K would only cause a decreasing inward tail.

Other currents may modify the dominant and subsidiary pacemaker potentials. For example, the Ca^{2+} current may be activated as DD approaches the threshold¹². However,

there would be no activation of the Ca^{2+} current if DD were not to depolarize the membrane to a suitable potential range.

In summary, DD is caused by a voltage- and time-dependent change of a current that had been modified by the previous AP. Thus, either the activation of I_K or the deactivation of I_f during the AP would cause a subsequent undershoot to the MDP. At the MDP, the deactivation of I_K in the dominant and the activation of I_f in the subsidiary pacemaker range lead to the subsequent DD. Whether one or the other mechanism is brought into play will depend on the diastolic range that the MDP attains. In the less negative (dominant) range, the deactivation of I_K causes the pacemaker potential, whereas in the more negative (subsidiary) range, the activation of I_f appears to be important¹⁰. What makes the difference in the pacemaker potential range is the inward rectifier current I_{K1} . In dominant cells, the inward rectifier I_{K1} channel is little expressed^{7,10} and therefore the diastolic potential is less negative than in subsidiary pacemakers.

2. Dual pacemaker mechanisms in SAN subsidiary pacemaker cells

The two pacemaker currents present in different diastolic ranges (dominant and subsidiary) can both be present in SAN subsidiary pacemaker cells: the subsidiary mechanism in the range of subsidiary DD and the dominant mechanism when the subsidiary cells are depolarized within the dominant pacemaker range. The presence of two pacemaker mechanisms can be demonstrated by depolarizing the cells by means of Cs^+ or high $[K^+]_o$.

In subsidiary pacemaker cells, Cs^+ (in addition to blocking I_p) blocks I_{K1} , thereby decreasing the membrane potential into the dominant pacemaker range and unmasking the dominant pacemaker mechanism¹³ which is less sensitive to Cs^+ ¹⁴. Cs^+ initially hyperpolarizes the cell membrane, but it does so by stimulating the Na^+-K^+ pump ("Na⁺ pump"), and not by blocking I_f (see below). Subsequently, Cs^+ depolarizes by blocking an outward current (presumably I_{K1} which is present in subsidiary cells¹⁰). Cs^+ unmasks a dominant DD at depolarized levels, because it does not block I_K ¹⁴. Thus, in SAN subsidiary cells there are Cs^+ -sensitive currents at more negative potentials (presumably I_f and I_{K1}) and a Cs^+ -insensitive current at less negative potentials (presumably I_K)^{13,14}.

High $[K^+]_o$ (which does not block I_K ¹⁵, increases I_f ¹⁶ and stimulates the Na^+ pump activity¹⁷), depolarizes subsidiary pacemaker cells, which then exhibit dominant-type APs. Adding Cs^+ causes a very small increase in MDP (in high $[K^+]_o$, the Na^+ pump activity is already stimulated), fol-

lowed by a persisting subsequent decrease (which could not be due to the block of the inward I_f). In preparations quiescent in high $[K^+]_o$, Cs^+ only depolarizes and can induce spontaneous discharge: the APs are followed by an undershoot and DD (Fig. 1), which can not involve the deactivation and activation (respectively) of I_f since 8 mM Cs^+ is present. In high $[K^+]_o$ plus norepinephrine, even 20 mM Cs^+ do not suppress and might increase the rate. During quiescence in acetylcholine or carbachol (I_f is blocked¹⁸), Cs^+ still transiently hyperpolarizes the resting potential. In zero $[K^+]_o$ (I_f is absent¹⁹) with or without carbachol, Cs^+ hyperpolarizes the quiescent membrane by stimulating the Na^+ pump. Cs^+ -induced hyperpolarization is reduced by ouabain¹³.

That the important factor in the induction of dominant DD in high $[K^+]_o$ is the depolarization induced is shown by the fact that Ba^{2+} (which, in contrast to high $[K^+]_o$, reduces K^+ conductance) also decreases the resting potential and allows the appearance dominant APs. Micromolar concentrations of Ni^{2+} do not suppress SAN discharge (and actually increase it), but nifedipine does so. In Tyrode solution, nifedipine slows, but does not stop the SAN²⁰. High $[Ca^{2+}]_o$ quickly induces spontaneous activity and low $[Ca^{2+}]_o$ stops it and markedly reduces ThV_{os} in driven preparations²¹.

The results suggest that discharge of dominant APs is due only to the dominant pacemaker current I_K at less negative potentials and that discharge is particularly sensitive to changes in Ca^{2+} current. Also, the results support the notion that I_f is not involved either in the dominant pacemaker mechanism or in the events leading to the initiation of spontaneous activity.

3. Block of different diastolic currents and sino-atrial node discharge

If there are two pacemaker mechanisms in the SAN that are activated in different diastolic ranges, the question arises as to whether either one is sufficient to maintain spontaneous discharge. This was studied by testing blockers of either pacemaker current²². In subsidiary pacemakers of rabbit and guinea pig isolated SAN, as usual, Cs^+ or high $[K^+]_o$ unmask the dominant DD at depolarized levels, but do not stop the SAN. In rabbit SAN, E4031 and d-sotalol (blockers of I_{Kr} , the fast component of I_K) do not stop discharge, but do so after block of subsidiary DD by Cs^+ or by high $[K^+]_o$ (the latter eliminates I_f by depolarizing the membrane outside the subsidiary pacemaker range).

In guinea pig SAN, in Tyrode solution E4031, d-sotalol or indapamide (a blocker of I_{Ks} , the slow component of I_K) do not stop SAN discharge. In the presence of Cs^+ or high $[K^+]_o$, indapamide (but not E4031 nor d-sotalol) stop the

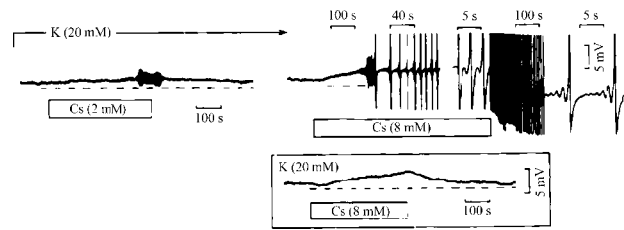


Fig. 1 Induction of pacemaker discharge by Cs^+ in SAN. SAN was quiescent in high $[K^+]_o$ and adding 2 mM Cs^+ caused only depolarization which at its peak induced oscillatory potentials (ThV_{os}). Increasing Cs^+ depolarized the resting potential more, induced larger ThV_{os} , which in turn initiated spontaneous discharge. The APs were followed by an undershoot to the MDP and subsequent diastolic depolarization, suggesting that neither the undershoot was not due to I_f deactivation nor DD to I_f activation. In the boxed inset, Cs^+ induced a very small hyperpolarization and a reversible depolarization. (Reprinted from *J Mol Cell Cardiol*, vol. 27, Sohn HG, Vassalle M. Cesium effects on dual pacemaker mechanisms in guinea pig sinoatrial node. pp. 563-577, Copyright 1995 with permission from Elsevier).

guinea pig SAN discharge. Ba^{2+} leads to stoppage of discharge both in Tyrode solution and in high $[K^+]_o$ or Cs^+ . Depolarization by blockers of DD unmask sinusoidal fluctuations (ThV_{os} , see below), which during recovery are responsible for resumption of discharge.

Thus, in rabbit and guinea pig SAN, the two pacemaker mechanisms (Cs^+ - and K^+ -sensitive subsidiary DD, and Cs^+ - and K^+ -insensitive dominant DD) can independently sustain discharge, but block of both mechanisms leads to quiescence. Abolition of dominant DD by blockers of I_K is consistent with a decay of I_K as the dominant pacemaking mechanism, I_{Kr} being more important in rabbit and I_{Ks} in guinea pig.

Purkinje Fibers

1. The pacemaker mechanism: I_{Kdd} versus I_f

In patients with complete atrio-ventricular block, the rate of discharge of idioventricular rhythm is 30/40 beats per minute. With an interval of about two seconds between APs, in Purkinje fibers DD lasts about 1500 ms, which is already an indication that the slope of DD is much less steep than in SAN. This suggests that the underlying pacemaker current may also be different.

In spontaneously active Purkinje fibers, suppressing DD by voltage-clamping the membrane at the MDP results in a time-dependent net inward current²³. The membrane conductance decreases during the pacemaker current, sug-

gesting that the current is net inward because of the decrease of a K^+ conductance. This conclusion is strengthened by the fact that the pacemaker current (I_{Kdd}) reverses past the potassium equilibrium potential E_K . The pacemaker current is partially activated at the resting potential and it is fully and quickly activated during the plateau²³. It was later shown that this K^+ pacemaker current has an activation range between -90 and -60 mV and undergoes inward rectification²⁴ and that the reversal potential shifts in different E_K ^{24,25}.

These conclusions were generally accepted until 1981, when it was proposed that the evidence supporting the I_{Kdd} hypothesis was invalid due to K^+ depletion in narrow extracellular spaces. Such a K^+ depletion current would account for the decrease in conductance during the pacemaker current, its pseudo-reversal and the shift of the reversal with different $[K^+]_o$ ^{16,26}. In support of this new proposal, when K^+ depletion was prevented by blocking I_{K1} with Ba^{2+} , the time-dependent current (called I_f) did not reverse any longer and the membrane conductance increased during the current^{16,18,26}. Cs^+ did not increase the instantaneous current on hyperpolarization, but blocked the time-dependent current during the hyperpolarizing step¹⁶. All these findings were interpreted to mean that Ba^{2+} prevented the masking of I_f by the K^+ depletion current and that Cs^+ specifically blocked the inward I_f (and not the outward I_{Kdd}).

There were several major problems with the correctness of the I_f hypothesis. One was whether K^+ depletion current occurred in the range of DD (which is positive to E_K). Another one is that Ba^{2+} blocks several K^+ currents and therefore there was the distinct possibility that it blocked the very current meant to be investigated. A third source of doubts was whether Cs^+ (which blocks several K^+ channels) is indeed a specific blocker of I_f .

Because of the major disagreement about the ionic current (I_{Kdd} versus I_f) underlying the pacemaker potential in Purkinje fibers, it becomes necessary to address the above issues in detail and to present pertinent evidence that might permit to find the reasons for the disagreement and to identify which of the two hypotheses is the correct one.

2. Lack of evidence for K^+ depletion in the voltage range of diastolic depolarization

Even if K^+ depletion in narrow extracellular spaces may occur during large hyperpolarization negative to E_K , the depletion current would have to be large enough not only to match I_f but actually to reverse it in order to account for the current reversal. Furthermore, K^+ depletion does not necessarily occur in the DD potential range, where the net

driving force for K^+ is outward (not inward).

One parameter of great interest in this regard is the change in membrane conductance during the pacemaker potential and current (the conductance should decrease during I_{Kdd} and increase during I_f). The membrane conductance does decrease during DD²⁷, but those results (while consistent with a decrease in a K^+ current and not with an increase in I_f) are complicated by the fact that the diastolic potential becomes less negative (increasing inward rectification of I_{K1}). Still, the membrane conductance decreases in the DD range even when the potential is kept clamped at a given value. In thin strands of quiescent Purkinje fibers²⁸, in the voltage range of DD, the membrane conductance decreases during the pacemaker current by 19.0%, a value similar to that found in spontaneously active Purkinje fibers clamped at the MDP (23.8%)²³. Small concentrations (2 mM or less) of Cs^+ do not block K^+ depletion¹⁶, yet Cs (2 mM) markedly reduces I_{Kdd} amplitude (79.8%) and the decrease in membrane conductance (89.0%) while reducing much less the initial instantaneous current (28.4%). If K^+ depletion were responsible for the decrease in membrane conductance, such a decrease would have persisted in spite of the near abolition of I_{Kdd} . In fact, if Cs^+ had suppressed I_f and the associated increase in membrane conductance, the decrease in conductance due to K^+ depletion should have been larger (and instead it was almost abolished).

To verify whether a small K^+ depletion occurs even at potentials positive to E_K , small concentration of Ba^{2+} were used to reduce I_{K1} and therefore possible K^+ depletion. If the time-dependent current on hyperpolarization was partially distorted by a K^+ depletion current, not only the instantaneous but also the time-dependent current should have changed. Ba^{2+} (0.05 mM) shifted the holding current in an inward direction, and decreased the instantaneous jump on hyperpolarization by -56.9% whereas the pacemaker current was hardly modified (-1.9%).

Therefore, Ba^{2+} substantially reduces I_{K1} but does not modify I_{Kdd} amplitude, suggesting that K^+ depletion plays little role in the current recorded in the DD voltage range. Increasing $[K^+]_o$ from 2.7 to 5.4 mM roughly doubles membrane conductance^{23,28}. Therefore, the higher $[K^+]_o$ should increase I_{K1} and K^+ depletion (if any) during the step: the possible decaying depletion current would decrease the net inward pacemaker current. In the higher $[K^+]_o$, the instantaneous current jump increased by 182.9%, whereas I_{Kdd} was little changed (+17.5%). Therefore, while higher K^+ conductance more than doubled the instantaneous current, the consequent depletion must have rather small (if any) since the pacemaker current was little affected.

To verify whether the same results would be obtained in 5.4 mM $[K^+]_o$ in the presence of Ba^{2+} , which would reduce the increase in K^+ conductance induced by the higher $[K^+]_o$, steps were applied in 5.4 mM $[K^+]_o$ in the absence and presence of low $[Ba^{2+}]_o$. The instantaneous current was decreased by Ba^{2+} (-41.6%), whereas I_{Kdd} slightly increased (+6.8%). Thus, the decrease of the instantaneous current was five fold greater than the increase in I_{Kdd} .

$[K^+]_o$ was increased to 10.8 mM to increase I_{K1} and therefore exaggerate K^+ depletion or accumulation. During a depolarizing step, the decay of the transient outward current I_{to} is associated with a decrease in membrane conductance. During a hyperpolarizing step negative to E_K , the current and the conductance decrease with time. Therefore, changes in conductance due to time-dependent currents appear to predominate over whatever opposite changes in conductance might be caused by changes in $[K^+]_o$. If the reversal of I_{Kdd} at negative potentials were only due to a depletion of $[K^+]_o$ which masks the increase in I_f , Cs^+ (by blocking the inward I_f) should increase the decay of the depletion current. In 10.8 mM $[K^+]_o$, 2 mM Cs^+ decreased the instantaneous jump by -33.4% and the reversed time-dependent current by -35.9% and reduced the decrease in conductance: apparently then, Cs^+ did not block an inward I_f since the current during the step did not become more outward (depletion current being no longer antagonized by I_f) nor did conductance decrease more (due to the elimination of the increase in conductance associated with I_f activation).

All these results²⁸ were interpreted as indicating that a time-dependent decrease in a K^+ conductance is the cause of DD, that K^+ depletion does not occur in the DD range (which is positive to E_K), and K^+ depletion does not invalidate previous findings.

3. Lack of specificity of the cesium block of the pacemaker current

An essential point here is that, if Cs^+ blocks I_f but not specifically, the block of a current by Cs^+ can not be taken as evidence that the blocked current is necessarily I_f . The basis for proposing that Cs^+ specifically blocks I_f was that a block of an outward pacemaker current should increase the instantaneous current jump at the beginning of a hyperpolarizing voltage step and such an increase does not occur¹⁶. However, this rationale is open to a degree of uncertainty. I_{Kdd} is already partially activated at the resting potential²³ and Cs^+ would block the pacemaker current at the holding potential prior to the hyperpolarizing step. In fact, in the steady state, Cs^+ shifts the holding current in an inward direction, as expected from a block of an outward

current²⁸.

In the absence of Cs^+ , on hyperpolarization, the instantaneous decrease of I_{Kdd} as a function of voltage (e.g., no I_{Kdd} at E_K) plus the increase in driving force for the Na^+ background current would result in an inward instantaneous current jump. In the presence of Cs^+ , the already partially blocked I_{Kdd} at the holding potential would decrease less on hyperpolarization than in control and thus the inward instantaneous current would be smaller. Therefore, the instantaneous current may decrease, not increase as proposed in the rationale for a specific Cs^+ block of I_f .

Cs^+ blocks also other K^+ currents. For example, Ba^{2+} induces a pacemaker potential and a pacemaker current in ventricular myocardial cells by a time- and voltage-dependent block of I_{K1} : this potassium pacemaker current is blocked by $Cs^{+29,30}$. Although this pacemaker current is different from I_{Kdd} , still the point is that Cs^+ blocks a current that is certainly a K^+ pacemaker current (no I_f in myocardial cell in the diastolic range)^{30,31}. Cs^+ also blocks radioactive K^+ fluxes in cardiac tissues³² and different K^+ currents in different tissues³³. It will be shown below that Cs^+ blocks also I_{Kdd} . Thus, Cs^+ block of I_f is not specific and a Cs^+ -block of a pacemaker current does not prove that it is I_f .

4. Mechanisms underlying hyper- and de-polarizing actions of Cs^+

Cs^+ increases the resting potential^{13,33-35} and decreases intracellular sodium activity (a_{Na}^i)³³ in Purkinje fibers. Both actions have been attributed to a Cs^+ block of I_f ^{36,37}. However, both actions could instead be due to a Cs^+ -induced stimulation of the activity³⁸ of the electrogenic Na^+ pump.

The latter explanation is supported by the following findings. Cs^+ -induced hyperpolarization is transient³³⁻³⁵ as expected from the fact that the stimulation of the Na^+ pump by Cs^+ is offset by the consequent decrease in a_{Na}^i . Increasing concentrations of Cs^+ decrease a_{Na}^i to a similar extent, but cause a smaller initial hyperpolarization and a larger subsequent depolarization. Cs^+ causes hyperpolarization and decreases a_{Na}^i also in myocardium³³, in spite of the absence of DD and of I_f at the resting potential in that tissue. The block of the pacemaker potential can be dissociated from the decrease in a_{Na}^i by means of tetrodotoxin administration (Cs^+ blocks DD, but decreases the already low a_{Na}^i little)³³. In fibers driven at fast rate there is no diastole, and therefore DD and the activation of I_f are not present: yet, Cs^+ decreases a_{Na}^i even more than when DD is present at slower rates³³. In zero $[K^+]_o$ (no I_f ¹⁹), Cs^+ hyperpolarizes and decreases a_{Na}^i in Purkinje and atrial myocardium. When the Na^+ pump is blocked by strophanthidin, Cs^+ fails

to modify the membrane potential and a_{Na}^i ³³. Rubidium also stimulates the Na^+ pump and blocks I_f ¹⁹, yet rubidium decreases a_{Na}^i , but decreases the resting potential in both Purkinje and myocardial fibers. All these results show that the block of I_f by Cs^+ is not specific and that the transient hyperpolarization by Cs^+ is accounted for by a stimulation of the electrogenic Na^+ pump and not by the block of I_f .

In another approach, the mechanisms of the hyperpolarizing and depolarizing actions of Cs^+ were studied in active and quiescent Purkinje fibers by means of a microelectrode technique in order to determine whether Cs^+ hyperpolarizes the membrane potential in a manner consistent either with the stimulation of the Na^+ pump activity³³ or with the block of I_f ¹⁶, and whether Cs^+ depolarizes in a manner consistent with a block of I_{Kdd} . It was found³⁵ that in active fibers Cs^+ (2 mM) inconsistently increases and then decreases the MDP, and markedly decreases DD. The increase and decrease in MDP induced by Cs^+ were also present in fibers driven at fast rate (no diastolic interval and no activation of I_p). In quiescent fibers, Cs^+ causes an initial hyperpolarization which subsides as a function of time. In the presence of Cs^+ , after a period of quiescence, driven action potentials are followed by very small undershoot, as it would be expected by the block of an outward pacemaker current activated during the AP. In zero mM $[K^+]_o$ (no I_f ¹⁹), the fibers depolarized at the plateau (where any residual I_f would be deactivated): Cs^+ induced a large and persistent (Cs^+ substituting for the K^+) hyperpolarization. These results also suggest that the hyperpolarization is due to the stimulation of an electrogenic Na^+ pump and not by the block of I_f .

Cs^+ suppresses spontaneous discharge not by preventing the depolarization from the MDP (as it would be expected from a block of I_p), but by preventing the attainment of the threshold through hyperpolarization and through a decrease in MDP and DD amplitude. In high $[K^+]_o$, Cs^+ causes a small inconsistent hyperpolarization (the Na^+ pump being already stimulated by high $[K^+]_o$) and a subsequent depolarization in quiescent fibers; and decreases MDP in driven fibers. When Cs^+ is increased to 4, 8 and 16 mM, the initial hyperpolarization is gradually smaller and the subsequent depolarization gradually larger. In addition, the AP of the depolarized fibers is not followed by an undershoot. In the presence of strophanthidin, Cs^+ hyperpolarizes less³⁵.

These results are also consistent with the conclusion that Cs^+ causes a transient hyperpolarization by stimulating the electrogenic Na^+ pump activity (and not by suppressing I_p) and blocks the pacemaker potential by blocking the undershoot, consistent with a Cs^+ block of a K^+

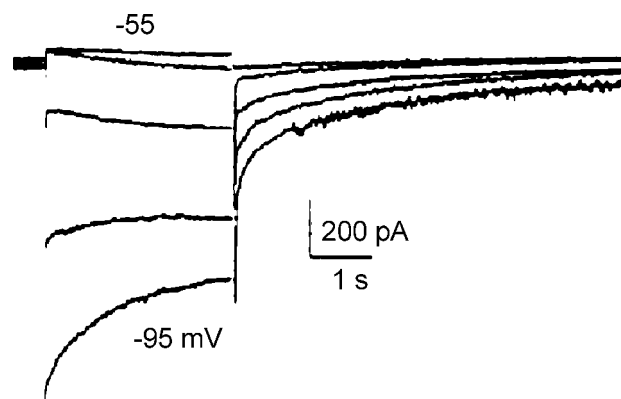


Fig. 2 Reversal of the pacemaker current I_{Kdd} in a single Purkinje cell in 5.4 mM K^+ Tyrode solution. The holding potential was -50 mV and a hyperpolarizing step was applied to -55, -65, -75, -85 and -95 mV. The increase in current was small at -55 mV and became larger at -65 and -75 mV. The current reversed at -85 mV (Reproduced from the Journal of General Physiology, 1995;106:559-578; Vassalle M, Yu H, Cohen IS. The pacemaker current in cardiac Purkinje myocytes, by copyright permission of The Rockefeller University Press).

pacemaker current.

5. K^+ depletion, Ba^{2+} and patch clamp experiments in single Purkinje cells

The results so far reviewed indicate that there is no evidence supporting the occurrence of an extracellular K^+ depletion in DD voltage range, the specificity of Cs^+ block of I_p and Cs^+ -induced hyperpolarization being due to a block of I_f . As for the proposal that Ba^{2+} unmasks I_f by preventing K^+ depletion (rather than by blocking I_{Kdd}), this issue was studied with the whole cell patch-clamp technique in single Purkinje cell. This approach has several major advantages. One is that there are no restricted intercellular spaces (and Purkinje cell do not have T tubules³⁹) and therefore it is possible to study the pacemaker current in the absence and in the presence of Ba^{2+} . This also allows the investigation of the effects of Ba^{2+} on the currents under study⁴⁰.

In 5.4 mM K^+ Tyrode solution, hyperpolarizing steps from the holding potential (V_h) = -50 mV result in a time-dependent current that has a threshold of -61 mV and reverses near E_K (I_{Kdd}) (Fig. 2). Decreasing $[K^+]_o$ from 5.4 mM to 2.7 mM shifts the reversal potential of I_{Kdd} near the more negative E_K . Increasing $[K^+]_o$ to 10.8 mM almost abolishes I_{Kdd} . Cs^+ (2 mM) markedly reduces I_{Kdd} at potentials positive and negative to E_K .

A point of major interest is that perfusion with 4 mM

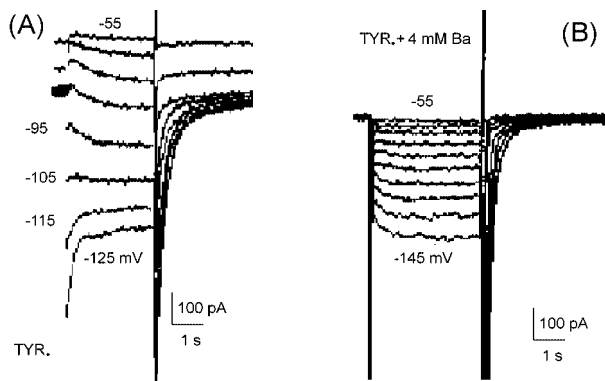


Fig. 3 $I_{K_{dd}}$ in the absence and I_f in the presence of Ba^{2+} . All panels were recorded from a Purkinje myocyte superfused with 2.7 mM $[K^+]_o$. The holding potential was -50 mV and hyperpolarizing pulses were applied to -55, -65, -75, -85, -95, -105, -115 and -125 mV in the absence (A) and to -55, -65, -75, -85, -95, -105, -115, -125, -135 and -145 mV in the presence of 4 mM Ba^{2+} (B). (Reproduced from the Journal of General Physiology, 1995;106:559-578; Vassalle M, Yu H, Cohen IS. The pacemaker current in cardiac Purkinje myocytes, by copyright permission of The Rockefeller University Press).

Ba^{2+} eliminates $I_{K_{dd}}$ in its usual range of potentials and unmasks I_f with a threshold of -88 mV (27 mV more negative than that of $I_{K_{dd}}$ in Tyrode solution) (Fig. 3). During more negative steps, I_f increases in size and does not reverse. High $[K^+]_o$ (10.8 mM) markedly increases and Cs^+ (2 mM) blocks I_f .

The slope conductance decreases during the decay of $I_{K_{dd}}$ in Tyrode solution (no Ba^{2+}) and increased during the activation of I_f in the presence of Ba^{2+} (Fig. 4). The time constant of the changes of the time-dependent currents is similar to that of the changes of slope conductance, suggesting that $I_{K_{dd}}$ was not contaminated by I_f in Tyrode solution and I_f was not contaminated by $I_{K_{dd}}$ in the presence of Ba^{2+} .

These results show that $I_{K_{dd}}$ does reverse near E_K and the reversal is not due to K^+ depletion, but could contain a component due to I_{K1} inactivation during hyperpolarization negative to E_K ; the slope conductance of $I_{K_{dd}}$ decreases in the absence of K^+ depletion at potentials positive to E_K where I_{K1} is unlikely to be inactivated. Ba^{2+} blocks $I_{K_{dd}}$ and unmasks I_f with a threshold some 25 mV negative to that of $I_{K_{dd}}$; Cs^+ decreases both $I_{K_{dd}}$ in the absence of Ba^{2+} and I_f in the presence of Ba^{2+} . Thus, in Purkinje cells the pacemaker current is due to a voltage- and time-dependent decrease in K^+ conductance⁴⁰, as initially proposed²³, which can be separated from hyperpolarization-activated inward I_f .

The discrepant interpretations of the pacemaker current in Purkinje fibers apparently resulted from three factors:

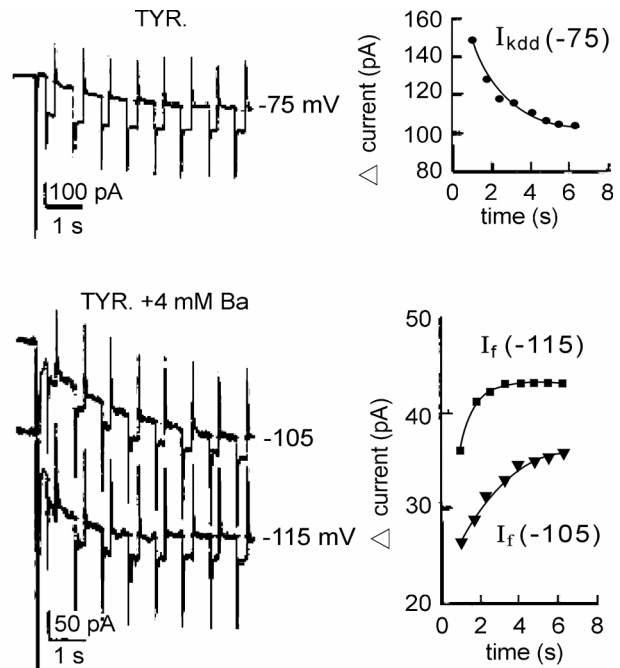


Fig. 4 Decrease in slope conductance during $I_{K_{dd}}$ in the absence of Ba^{2+} and increase in conductance during I_f in the presence of Ba^{2+} . In Tyrode solution (no Ba^{2+} , top panels), the decrease in amplitude of the current steps superimposed on the test step to -75 mV shows that the slope conductance decreases during $I_{K_{dd}}$. In the presence of 4 mM Ba^{2+} (bottom panels), the increase in amplitude of the current steps superimposed on the test steps to -105 and -115 mV show that the slope conductance increases during the activation of I_f . The graphs show the changes in the amplitude of the small current pulses during $I_{K_{dd}}$ (top graph, dots) in Tyrode solution and during I_f at -105 (triangles) and -115 (squares) mV in the presence of Ba^{2+} (bottom graphs). (Reproduced from the Journal of General Physiology, 1995;106:559-578; Vassalle M, Yu H, Cohen IS. The pacemaker current in cardiac Purkinje myocytes, by copyright permission of The Rockefeller University Press).

the confounding effect of K^+ depletion at potential negative to E_K , where the depletion current was assumed to be large enough not only to mask the activating I_f , but also to induce a spurious reversal of the time-dependent current; the use of Ba^{2+} to block I_{K1} and therefore K^+ depletion; the belief that Cs^+ was a specific blocker of I_f .

The various approaches that we adopted show that positive to E_K there is little, if any, K^+ depletion; no $I_{K_{dd}}$ is seen in presence of Ba^{2+} because Ba^{2+} blocks it; and Cs^+ is not a specific blocker of I_f since, it blocks also $I_{K_{dd}}$. The results with Purkinje strands provide no evidence that the pacemaker potential is due to I_f and instead suggest that $I_{K_{dd}}$ is the pacemaker current. By using single Purkinje myocytes,

we avoided the possibility of a K^+ depletion on hyperpolarization. Therefore, we could separately study the pacemaker current in the absence of Ba^{2+} and effects of Ba^{2+} on $I_{K_{dd}}$. The results show that in Tyrode solution only $I_{K_{dd}}$ is generally seen at diastolic potentials and that Ba^{2+} abolishes it, thus unmasking I_f at a potential usually negative to the MDP. Therefore, in Purkinje fibers the decay of $I_{K_{dd}}$ appears to play the major role in the pacemaker potential.

One point need to be clarified, namely, how the decay of the outward $I_{K_{dd}}$ can result in a diastolic inward current. Naturally, the answer is that DD is due to a net inward current, resulting from a time-independent inward current (background Na^+ current) and a decaying time-dependent outward K^+ current ($I_{K_{dd}}$). The pacemaker current has to be a voltage- and time-dependent current. The decay of a time-dependent outward current in the presence of an inward background current causes a “time-dependent net inward current”. During DD, the net current becomes inward with time only because the outward pacemaker current deactivates. Some view the time-independent “background” current as a possible pacemaker current. However, a time-independent inward background current would not cause (as it does not in myocardial fibers) a slow decline in diastolic pacemaker potential. DD can be caused only by a time-dependent current in the DD range in the presence of a time-independent background current.

It should be noted that I_f is a mixed Na^+ and K^+ pacemaker current¹⁶: during DD, any increase in conductance of the I_f channel would cause progressively more K^+ to leave the cell, since the membrane potential is positive to E_K and more so as DD progresses. The outward flow of K^+ through the I_f channel would oppose DD.

The relationship between the AP and the pacemaker current would be as follows²³. $I_{K_{dd}}$ is partially activated at the resting potential. During the AP, $I_{K_{dd}}$ fully activates and during phase 3 repolarization the increased membrane conductance induced by the $I_{K_{dd}}$ channel allows the potential to approach E_K . However, the slow deactivation of the $I_{K_{dd}}$ channel at negative potentials causes DD in the presence of a time-independent inward background Na^+ current. In lower $[K^+]_o$, the undershoot and DD are larger, as the activation of $I_{K_{dd}}$ during the AP allows the membrane to approach the more negative E_K ^{23,28}.

OSCILLATORY POTENTIALS AND THE PACEMAKING PROCESS

As mentioned in the INTRODUCTION, diastolic depolarization can not be equated with spontaneous discharge, since in quiescent fibers; a driven AP is followed by DD

but no spontaneous discharge. Thus, DD is only a component (albeit essential) of the pacemaking process. Both in the SAN and in Purkinje fibers, there are afterpotentials (V_{os}) and prepotentials (ThV_{os}) which also appear to play an obligatory role in spontaneous discharge under normal or abnormal conditions. ThV_{os} appear to participate in the discharge of both the dominant pacemakers of SAN and Purkinje fibers. V_{os} also seems to participate in dominant pacemaker discharge in SAN under normal conditions, and it participates in pacemaker discharge of Purkinje fibers under certain abnormal conditions (calcium overload).

As mentioned above, DD is an afterpotential (it follows the action potential) and therefore prepotentials (ThV_{os}) are needed for the initiation of spontaneous discharge in a quiescent pacemaker. In addition, it is possible to demonstrate that ThV_{os} is the link between DD and threshold for the upstroke. Therefore, ThV_{os} are also necessary for the maintenance of spontaneous discharge, both in SAN and in Purkinje fibers. V_{os} contributes to fast discharge in different ways (see below).

A. Sino-Atrial Node

In SAN pacemakers, under normal conditions no oscillatory potentials are seen. Therefore, naturally enough, it is generally believed that the pacemaking process involves only the pacemaker current. However, the picture changes substantially when $[K^+]_o$ is increased, since DD become smaller and less steep until it misses the threshold. When it does so, diastolic oscillations become visible and distinct one from the other and from initial diastolic depolarization (DD_1).

As usual, when $[K^+]_o$ is increased, the MDP decreases and all APs assume dominant-like configuration (slow responses with U-shaped DD). High $[K^+]_o$ then unmasks the two types of oscillatory potentials mentioned, namely, V_{os} (which is obligatorily superimposed on DD_1) and ThV_{os} (which can occur at any time during diastole)^{13,20,21,27,41}. In high $[K^+]_o$, SAN abruptly slows down when ThV_{os} fail to attain the threshold: it is this failure that unmasks ThV_{os} and their role in the maintenance of spontaneous discharge. However, this does not lead to a permanent SAN arrest, since the subsequent DD_2 enters a less negative voltage range (“oscillatory zone”) and ThV_{os} appear again. They gradually increase in size and attain the threshold for the AP, indicating their obligatory role in the initiation of spontaneous discharge (Fig. 5). As ThV_{os} occur later during diastole, they become separated from V_{os} which remains superimposed on DD_1 . If ThV_{os} consistently miss the threshold, they decrease in size, disappear and quies-

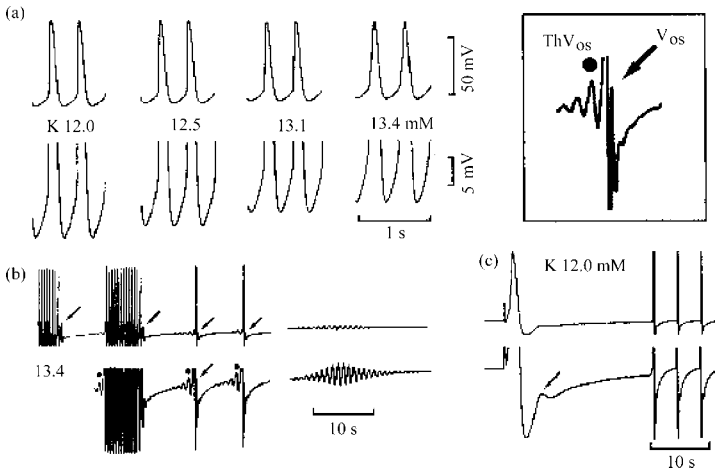


Fig. 5 High $[K^+]_o$ depolarizes SAN and unmasks diastolic oscillations.

In (a), the SAN was superfused in progressively higher $[K^+]_o$ as indicated by numbers between the traces at normal and at higher gain. In the fourth (a) panel, an inflection is seen during the upstroke. At the beginning of (b) the threshold was missed (first oblique arrow) revealing an oscillation that quickly decreased (subsequent 3 arrows). In the higher gain lower trace, as DD continued, increasing oscillations appeared (ThV_{os}, dots) which led to the resumption of a slower rhythm. When ThV_{os} consistently missed the threshold (last b panel), they decreased in size and SAN became quiescent. The ThV_{os} and V_{os} labeled with a dot and an arrow are shown at higher gain in the boxed inset. In (c), the SAN was driven and the arrow points to a V_{os}. (Reprinted from J Mol Cell Cardiol, vol. 29, Kim EM, Choy H, Vassalle M. Mechanisms of suppression and initiation of pacemaker activity in guinea pig sino-atrial node superfused in high $[K^+]_o$, pp. 1433-1445, Copyright 1997 with permission from Elsevier).

cence follows. If the quiescent SAN is driven, the AP may be followed by V_{os} in the absence of ThV_{os} (Fig. 5c). These findings elicit a number of substantial questions, such as the relationship between V_{os}, ThV_{os}, DD and the APs or the mechanism by which ThV_{os} re-increased during DD₂ to actually attain the threshold.

That the dominant diastolic depolarization is due to a fusion V_{os}, ThV_{os} and the early diastolic depolarization (DD₁) can be inferred from the fact that, during continuing high $[K^+]_o$ perfusion, V_{os} and ThV_{os} become separated, with V_{os} following the AP and ThV_{os} appearing later during DD (Fig. 5b). Typically, the AP induced by ThV_{os} is followed by a V_{os} superimposed on DD₁ and by the suppression of ThV_{os}. The suppression of ThV_{os} by the AP is due to the undershoot to the MDP which is negative to the oscillatory zone. This is shown by the fact that if sub-threshold ThV_{os} are continuously present in a quiescent SAN, a spontaneous or a driven AP leads to the temporary suppression of ThV_{os}. ThV_{os} appear again and grow in amplitude during DD₂ because the inhibition due to the hyperpolarization to

the MDP is gradually removed as DD₂ progresses into the oscillatory zone. When ThV_{os} become large enough, they attain the threshold and initiate a slow discharge. If $[K^+]_o$ is further increased, the size of both oscillatory potentials continues to decrease and the smaller ThV_{os} consistently miss the threshold and SAN becomes quiescent⁴¹.

On reducing high $[K^+]_o$, ThV_{os} reappear, increase in size and initiate spontaneous discharge. As they occur progressively earlier during DD, they eventually fuse with V_{os}: at that stage, DD appears to continue directly into the upstroke (U-shaped DD) and the oscillations are no longer seen. That V_{os} and ThV_{os} are unmasked (rather than induced) by high $[K^+]_o$ is shown by the fact that a stepwise increase in $[K^+]_o$ reduces the amplitude of the oscillatory potentials, which is inconsistent with high $[K^+]_o$ inducing them. This is confirmed by the fact that during recovery in Tyrode solution, size and slope of V_{os} and of ThV_{os} increase markedly and cause a faster discharge, at which time they become no longer visible. As APs assume the subsidiary configuration, their DD (no longer U-shaped) abruptly terminates into the upstroke⁴¹.

Since ThV_{os} are temporarily suppressed after dominant APs because the MDP is negative to the oscillatory zone, it is not surprising that the U-shaped DD (due to the attainment of the threshold by the oscillatory potentials superimposed on DD₁) should not be present in the subsidiary diastolic range. The presence of ThV_{os} in the dominant and their absence in the subsidiary range may be a major characteristic that differentiate dominant from subsidiary pacemaker discharge⁴¹.

As for the factors that are involved in the genesis of V_{os} and ThV_{os}, Ca²⁺ seem to play an important role. In high $[K^+]_o$, increasing $[Ca^{2+}]_o$ increase size and slope of V_{os} and of ThV_{os}, which in turn restore or accelerate discharge. In contrast, low $[Ca^{2+}]_o$ abolishes V_{os} and ThV_{os} and causes SAN arrest^{21,41}. However, it appears that it is $[Ca^{2+}]_i$ and not $[Ca^{2+}]_o$ that is important for both V_{os} and ThV_{os}, since overdrive has the same effects as high $[Ca^{2+}]_o$ and millimolar concentrations of Ni²⁺ stops the SAN as low $[Ca^{2+}]_o$ does: during overdrive and the exposure to Ni²⁺, $[Ca^{2+}]_o$ is normal although $[Ca^{2+}]_i$ should change in opposite directions. Apparently, ThV_{os} are unrelated to the calcium sparks that appear during the late DD^{42,43} and that are caused by I_{Ca,T} liberating pockets of Ca²⁺ which activate the Na/Ca exchange⁴², since micromolar concentrations of Ni²⁺ not only do not stop the SAN but in fact increase the rate^{21,41}.

In other cardiac tissues, V_{os} appears when the sarcoplasmic reticulum (SR) becomes Ca^{2+} overloaded and disappears when the SR function is impaired⁴⁴. Ryanodine (a blocker of SR Ca^{2+} handling⁴⁵) eliminates V_{os} and markedly slows or stops discharge. Ryanodine also eliminates ThV_{os} , but it is not known whether this action is indirect, due to the smaller DD ⁴¹. Cs^+ induces¹³ (Fig. 1) or accelerates spontaneous discharge as ThV_{os} increase in size and reach the threshold sooner²⁰. Therefore, ThV_{os} and initiation of activity in SAN do not appear to involve I_f which is blocked by Cs^+ ^{14,16}.

These results show that ThV_{os} and V_{os} are separate voltage oscillations that play an obligatory role in the initiation and maintenance of SAN discharge, V_{os} by increasing the size and the slope of DD_1 and ThV_{os} by attaining the threshold in the dominant pacemaker range, either by gradually increasing during DD_2 at slow rates or by fusing with V_{os} at fast rates⁴¹.

B. Purkinje Fibers

Also in this tissue, ThV_{os} play an obligatory role in the initiation and maintenance of spontaneous activity. In vitro, in Purkinje fibers superfused at a physiological $[K^+]_o$ (4-5 mM), stoppage of drive is followed by a diastolic depolarization that reaches a steady resting potential (Fig. 6A)⁴⁶. When $[K^+]_o$ is sufficiently lowered (to ≤ 3 mM), the resting potential begins to oscillate and ThV_{os} rapidly increase in amplitude to a comparable extent above and below the original potential level and intermittently reach the threshold (Fig. 6B). As in the SAN, if ThV_{os} consistently miss the threshold, they decrease in size and spontaneous discharge ceases (Fig. 6C). Instead, if $[K^+]_o$ is suitably low, they originate progressively sooner during diastole until they fuse with early diastolic depolarization. At that stage, diastolic depolarization appears to proceed from the maximum diastolic potential to the threshold and ThV_{os} are no longer apparent^{27,46,47}.

As in SAN, the upward swing of last part of DD appears to be the depolarizing phase of a ThV_{os} . Thus, on exposure to higher $[K^+]_o$, the upward curvature that precedes the upstroke becomes much less pronounced and (when the threshold is missed) a ThV_{os} is unmasked (see Fig. 7 in Ref. 48). When ThV_{os} gradually decrease in size, the resting potential stabilizes at a value in between the maximum diastolic potential and threshold^{27,46,47}. Therefore, even during regular discharge, ThV_{os} are the link between DD and threshold, thereby contributing to the maintenance of spontaneous activity. What DD does is to bring the membrane potential into the oscillatory zone, where the activation of the depolarizing phase of a ThV_{os} attains the threshold.

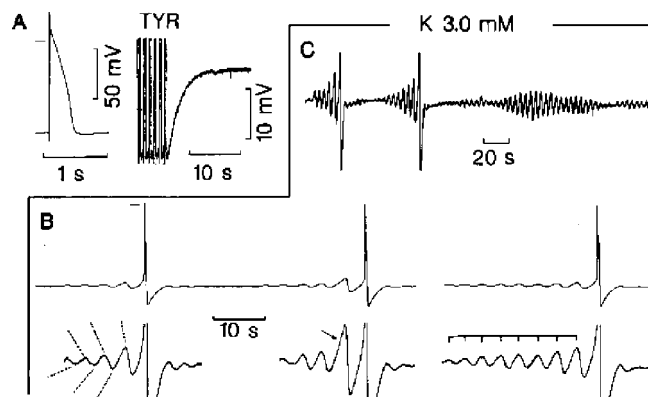


Fig. 6 Initiation of spontaneous activity by ThV_{os} in Purkinje fibers. In panel A, the traces were recorded in Tyrode solution ($[K^+]_o = 4$ mM). The fiber was driven at 60/min and, when the drive was interrupted, DD proceeded to the resting potential and the fiber remained quiescent. In panel B, $[K^+]_o$ was decreased to 3 mM and ThV_{os} appeared that led to spontaneous APs. In panel C, the higher gain trace shows that the AP induced by increasing ThV_{os} was followed by the suppression of ThV_{os} which re-increased during the late DD . When ThV_{os} missed the threshold, quiescence followed. (Reproduced from Spiegler P, Vassalle M. Role of voltage oscillations in the automaticity of sheep cardiac Purkinje fibers. *Can J Physiol Pharmacol* 1995;73:1165-1180. By permission of NRC Research Press)

In contrast to DD , the depolarizing phase of ThV_{os} appears to be somehow related to Na^+ entry, since tetrodotoxin (TTX) abolishes the discharge of Purkinje fibers by suppressing the late (but not the early) part of DD (see Fig. 2 in Ref. 49). The selective sensitivity of the upswing to TTX shows that this depolarizing potential is caused by a mechanism different from that of DD . In Purkinje fibers, at the time when TTX stops spontaneous discharge by suppressing ThV_{os} , APs followed by DD can still be elicited by electrical stimuli⁴⁶ showing that the stoppage is due neither to inexcitability nor to the suppression of DD .

As mentioned above, in low $[K^+]_o$, during successive ThV_{os} , the slope as well as amplitude of depolarizing and hyperpolarizing phases gradually increases (see dashed lines in Fig. 6B). This oscillatory behavior of ThV_{os} is not shared by DD : the decay of the pacemaker current I_{Kdd} is exponential and the pacemaker current does not oscillate^{23-25,40}. The hyperpolarization-activated I_f also does not oscillate^{16,19}. The pre-potentials ThV_{os} that occur at the resting potential are unlikely to be related to the after-potential V_{os} that may be superimposed on early DD . In fact, in Purkinje fibers, V_{os} are usually present only under conditions of calcium overload^{50,51}.

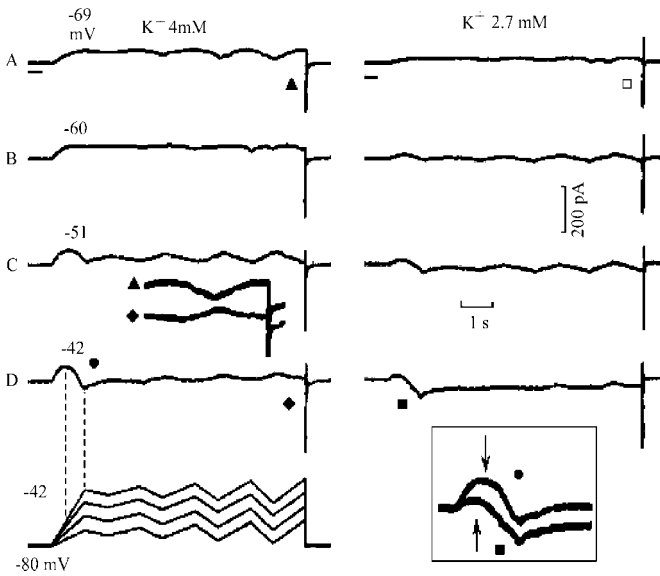


Fig. 7 Effects of ramps with a different slope and magnitude on I_{Na3} in normal and in low $[K^+]_o$ in a single Purkinje cell. The left hand traces were recorded in 4 mM $[K^+]_o$ and the right hand traces in 2.7 mM $[K^+]_o$. V_h was -80 mV and slope and magnitude of initial depolarizing ramp were progressively increased (11, 20, 29, 38 $mV s^{-1}$) and then oscillatory ramps were applied to the different potential levels (see protocol at the bottom and the numbers above the respective traces). The vertical dashed lines are drawn between beginning and end of the negative slope region and the corresponding voltages. Part of the traces labeled by a triangle and a diamond are juxtaposed between the C and D panels and show the reversal of current direction. Empty square marks the reversal of current direction in the lower with respect to the higher $[K^+]_o$. Gray areas emphasize the decay of I_{Na3} . Boxed inset shows the negative slope in 4 mM $[K^+]_o$ (dot) and in 2.7 mM $[K^+]_o$ (square). The arrows point to the negative slope and inward shift of the onset of the negative slope in the lower $[K^+]_o$. (Reproduced from Rota M, Vassalle M. Patch-clamp analysis in canine cardiac Purkinje cells of a novel sodium component in the pacemaker range. *J Physiol* (London) 2003;548.1:147-165, by permission of The Physiology Society).

Therefore, both Purkinje fibers and sino-atrial node share the ThV_{os} mechanism for the initiation and maintenance of rhythmic discharge. In SAN, V_{os} can contribute to spontaneous discharge in two ways: it steepens the slope of DD_1 , thereby accelerating spontaneous discharge; it can attain the oscillatory zone thereby initiating ThV_{os} ⁴¹. In Purkinje fibers, V_{os} also steepens DD_1 and can enter the oscillatory zone, but it is generally present when the SR is Ca^{2+} overloaded^{28,50,51}.

C. Mechanisms of the Oscillatory Potentials

The fact that ThV_{os} and V_{os} are present both in the sino-atrial node and in Purkinje fibers does not necessarily mean that they are caused by the same currents. That the currents might be different is suggested by the fact that the voltage range (oscillatory zone) at which they appear is much more negative in Purkinje fibers than in SAN. In both tissues, much is to be learned about the mechanisms underlying ThV_{os} , but some information is available about ThV_{os} in Purkinje fibers and this will be considered first.

1. ThV_{os} in Purkinje fibers

The fact that TTX abolishes the last part of DD as well as ThV_{os} suggests that a sodium current is involved. In order to account for the final upward swing that attains the threshold for the upstroke, this Na^+ component would have to inactivate slowly. We investigated this putative Na^+ current and its possible contribution to the depolarizing phase of ThV_{os} by means of patch voltage- and current-clamp techniques in single Purkinje cells⁵², thereby avoiding the complications associated with ion depletion and accumulation in narrow extracellular spaces.

The results obtained show that on depolarization there is a marked inward rectification which begins at the resting potential and is associated with a fall in slope conductance which reaches a minimum at about -60 mV. At that potential, the I-V relation undergoes a region of negative slope which appears to be mainly caused by a slowly inactivating Na^+ current (labeled I_{Na3}). I_{Na3} is an inward current that activates quickly and inactivates slowly during steps negative to the threshold for the fast sodium current I_{Na} . It is present also during slow depolarizing ramps that fail to activate I_{Na} . Its voltage-dependence is such that during depolarizing ramps, I_{Na3} appears at a threshold of ~ -60 mV and disappears at ~ -35 mV. I_{Na3} is time-dependent, since it is larger during faster depolarizing ramps (greater channel availability), and it is smaller or absent during slow depolarizing ramps or during repolarizing ramps (and therefore is different from the window current).

During faster ramps, I_{Na} appears superimposed on I_{Na3} , the latter current resuming its usual patterns after the quick inactivation of I_{Na} . Once initiated during a ramp, I_{Na3} slowly decreases as a function of time during the subsequent steady potential, as the channel inactivates over a period of seconds. During the negative slope, I_{Na3} is associated with a progressive increase in slope conductance and with a reversal of the polarity of the superimposed pulse currents (which is due to the negativity of the slope). During oscillatory ramps at less negative potentials, the current reverses direction, presumably because I_{Na3} is activated in

the negative slope region.

The role of Na^+ as the carrier of I_{Na3} is indicated by the fact that this current is markedly reduced by tetrodotoxin and lidocaine. In contrast, I_{Na3} is little affected by Cs^+ and Ba^{2+} , which, instead, markedly reduce the initial current during depolarizing ramps. It is of considerable interest that low $[\text{K}^+]_o$ (which initiates spontaneous discharge through ThV_{os}) increases the amplitude of I_{Na3} and shifts its activation potential to more negative values (Fig. 7). Reciprocally, high $[\text{K}^+]_o$ (which suppresses ThV_{os} and spontaneous discharge) has opposite effects on I_{Na3} . In voltage clamp mode, the results are consistent with the activation of I_{Na3} being responsible for the gradual transition between diastolic depolarization and the upstroke.

The conclusions suggested by these results are that within the pacemaker range there are two factors that are important contributors to spontaneous discharge. One is a voltage- and K^+ -dependent decrease in K^+ conductance on depolarization from the MDP, and the other (beginning at -60 mV) is the activation of a voltage- and time-dependent inward Na^+ component (I_{Na3}) with slow inactivation kinetics. In spontaneously discharging fibers, the decrease in slope conductance as a function of voltage (inward rectification of I_{K1}) would enhance the effects of the pacemaker current (time-dependent decrease of I_{Kdd}) thereby steepening DD, whereas I_{Na3} would permit the attainment of the threshold. The mechanisms by which low $[\text{K}^+]_o$ initiates spontaneous discharge in Purkinje fibers include a K^+ -dependent fall of the slope conductance in a critical range and a negative shift in the activation potential of a larger I_{Na3} .

2. ThV_{os} in the sino-atrial node

Although in SAN the behavior of ThV_{os} is quite similar to that in Purkinje fibers, the current responsible for the depolarizing phase of ThV_{os} is unlikely to be the same. Thus, the dominant pacemaker range in the SAN is far less negative than that of Purkinje fibers. In addition, (in contrast to Purkinje fibers) SAN dominant APs are little affected by TTX^{7,48}. Already it is known that Ca^{2+} entry contributes to the last part of DD¹². Furthermore, high $[\text{Ca}^{2+}]_o$ increases ThV_{os} and markedly enhances SAN discharge and low $[\text{Ca}^{2+}]_o$ has the opposite effect^{20,21,41}. Therefore, Ca^{2+} is likely to play in the ThV_{os} of the SAN the role that I_{Na3} plays in the ThV_{os} of Purkinje fibers.

3. V_{os} in Purkinje fibers

The mechanism underlying V_{os} has been extensively studied in Purkinje fibers and appears related to Ca^{2+} overload of the SR^{34,44,50,51,53-59}. In brief outline, under normal conditions, I_{Ca} releases Ca^{2+} from the SR in systole.

In order to maintain intracellular Ca^{2+} homeostasis, the Ca^{2+} that enters the cell through the slow channel in systole has to be extruded from the cell through the Na^+ - Ca^{2+} exchange in diastole. Instead, the Ca^{2+} released by the SR in systole is again taken up from the cytoplasm by that structure in diastole.

When the cytoplasmic Ca^{2+} load abnormally increases (e.g., high $[\text{Ca}^{2+}]_o$, toxic doses of digitalis, etc.) more Ca^{2+} is taken up by the SR during relaxation. However, when the SR becomes Ca^{2+} overloaded, apparently it becomes unstable. One possibility that has been proposed is that Ca^{2+} -induced Ca^{2+} release from the SR occurs not only during systole (as under normal conditions) but also in diastole, caused by the re-uptake of Ca^{2+} into the SR during the relaxation of the myofilaments⁵¹. This would account for the transient (oscillatory) release of Ca^{2+} during early diastole. This diastolic release is oscillatory (it starts, peaks and decays), since it would be triggered by the re-uptake of Ca^{2+} at the beginning of diastole.

The oscillatory release of Ca^{2+} induces at the same time an aftercontraction and an afterdepolarization (V_{os}). V_{os} is due to the fact that a new release of Ca^{2+} during diastole from the SR into the cytoplasm stimulates its extrusion through the Na^+ - Ca^{2+} exchange. Since the exchange is electrogenic (3 entering Na^+ are exchanged for 1 extruded Ca^{2+}) an inward current is created, which is oscillatory because the diastolic release of Ca^{2+} from the SR is oscillatory. If V_{os} is large enough, it may attain the threshold and initiates an AP or a train of APs. This sequence of events would account also for the fact that V_{os} occurs obligatorily after the AP (which induces a contraction whose relaxation may initiate the extra release of Ca^{2+}) and is superimposed on DD₁, reflecting the triggering of the extra Ca^{2+} by the relaxation process.

4. V_{os} in the sino-atrial node

While the above events may account for the arrhythmias induced by V_{os} in Purkinje fibers, the question arises as to why V_{os} should be present in the SAN under normal circumstances, and even in high $[\text{K}^+]_o$, which decreases Ca^{2+} influx¹⁵ and force of contraction²⁰⁻²². Two factors might contribute to overload the SR of dominant pacemaker cells⁴¹. One is that in SAN the SR is sparse⁶⁰ and therefore it could be Ca^{2+} -overloaded even under normal conditions. Another possible factor is that the driving force for Ca^{2+} extrusion might be smaller in SAN dominant pacemakers, because their diastolic potential is less negative than that in other cardiac tissues (decreased driving force for Ca^{2+} extrusion). Therefore, Ca^{2+} would tend to accumulate in the SR and this might explain the fact that

V_{os} is present even after the first AP elicited in a quiescent SAN⁴¹.

In direct support of the relation between V_{os} and Ca^{2+} load is the fact that V_{os} increases when Ca^{2+} load increases and decreases when Ca^{2+} load decreases^{21,41}. In addition, in high $[K^+]_o$, driving the SAN at rates in excess of 60/min increases contractile force⁶¹ through an accumulation of intracellular calcium, apparently because diastole is too short for the extrusion of the Ca^{2+} entering the cells during drive. After a period of fast drive, contractile force returns to control value only after long pauses⁶¹.

As shown in Purkinje fibers, V_{os} follows the AP because the underlying oscillatory current (I_{os} or I_{tr}) appears only after a depolarization is large enough to cause Ca^{2+} entry in the cell^{28,51}. A previous contraction is needed for the relaxation to initiate the oscillatory release of Ca^{2+} from the Ca^{2+} overloaded SR. I_{os} is also increased by high $[Ca^{2+}]_o$ as well as by repetitive depolarizing steps and by norepinephrine⁵¹. That the depolarizing event superimposed on DD_1 is V_{os} (and not ThV_{os}) (apart from the obligatory association of V_{os} with the AP and the inhibition of ThV_{os} by the AP) is indicated by the fact that typically the oscillations of V_{os} (if multiple) decrease whereas those of ThV_{os} increase.

CONCLUSIONS

The evidence reviewed suggests the following general conclusions. Spontaneous discharge necessitates diastolic depolarization as the mechanism by which the membrane potential decreases from the maximum diastolic potential to the oscillatory zone. And it necessitates ThV_{os} as the mechanism by which the membrane potential attains the threshold.

DD is determined a time-dependent change in conductance that leads to a net inward current. This can be accomplished during diastole either by a decreasing outward K^+ current or by an increasing inward Na^+ current. It appears the different cardiac pacemakers use different pacemaker currents. In SAN, the dominant pacemaker mechanism appears to be the decay of the delayed rectifier current I_K , whereas the subsidiary mechanism appear to be I_f activation¹⁰. These two mechanisms may be present in the same cell, the diastolic potential range determining which mechanism is actually brought into play. Thus, in subsidiary pacemaker cells the subsidiary mechanism is substituted by the dominant if the diastolic range becomes less negative^{10,20}. In Purkinje fibers, the pacemaker mechanism is due to the decay of the K^+ current I_{Kdd} ^{9,23,27,40}.

The advantages of these arrangements are several. In SAN, either of the two pacemaker mechanisms can sustain

spontaneous discharge²², which in itself is a safety mechanism. Furthermore, the two mechanisms being different¹⁰, they are not affected by the same procedures²² and this too is a safety mechanism. For example, hyperkalemia (by depolarizing subsidiary pacemaker cells) eliminates I_f but it does not suppress I_K ; and the subsidiary pacemaker depolarization decreases the electrotonic drag on SAN dominant pacemakers.

The different pacemaker current in Purkinje fibers²³ decays more slowly than that the dominant pacemaker current¹⁰ and this prevents Purkinje fibers from competing with SAN under normal circumstances, a safety mechanism which is enhanced by overdrive suppression^{1,2}.

The existence of an oscillatory zone in the voltage range of the resting potential appears to be an obligatory requirement for initiation of spontaneous activity in both SAN^{20,21,41} and Purkinje fibers^{46,47,52}. In addition, the depolarizing phase of ThV_{os} appears to be the link between DD and the threshold for the AP upstroke and therefore to be indispensable for spontaneous discharge of dominant SAN pacemakers^{20,21,41} and of Purkinje fibers^{46,47,52}.

The depolarizing phase of ThV_{os} in Purkinje fibers is due the slowly inactivating I_{Na3} ⁵², whereas in SAN dominant pacemaker cells is likely to be due to Ca^{2+} entry¹². V_{os} appear to contribute to fast discharge of SAN under normal circumstances⁴¹ and to that of Purkinje fibers under abnormal circumstances^{53,54}. Under abnormal circumstances (e.g., marked depolarization due to low $[K^+]_o$ ⁴⁷ or digitalis intoxication^{53,54}) also in Purkinje fibers the decay of I_K may lead to fast discharge at depolarized level.

ACKNOWLEDGMENTS

The original work cited in this review was supported by grants from N.I.H (National Heart and Lung Institute) and the American Heart Association, New York Affiliate.

REFERENCES

1. Vassalle M. Electrogenic suppression of automaticity in sheep and dog Purkinje fibers. *Circ Res* 1970;27:361-377.
2. Vassalle M. The relationship among cardiac pacemakers: overdrive suppression. *Circ Res* 1977;41:269-277.
3. Abete P, Ferrara N, Rengo F, Vassalle M. Mechanisms of lidocaine actions on normal and abnormal rhythms in canine cardiac tissues in vivo and in vitro. *Clin Exper Pharmacol Physiol* 1991;18:179-191.
4. Brooks McC, Lu HH. The Sinoatrial Pacemaker of

- the Heart. Springfield, IL: Thomas, 1972:68-69.
5. Ophhof T, de Jonge B, Mackaay AJ, Bleeker WK, Masson-Pévet M, Jongsma HJ, Bouman LN. Functional and morphological organization of the guinea pig sinoatrial node compared with the rabbit sinoatrial node. *J Mol Cell Cardiol* 1985;17:549-564.
 6. Lipsius SL, Vassalle M. Characterization of a two-component upstroke in the sinus node subsidiary pacemakers. In: Bonke FIM, ed. *The Sinus Node. Structure, Function and Clinical Relevance*. Hague: Nijhoff, 1978:233-244.
 7. Irisawa H, Brown HF, Giles W. Cardiac pacemaking in sinoatrial node. *Physiol Rev* 1993;73:197-227.
 8. Noble D. Ionic mechanisms in cardiac electrical activity. In: Zipes DP, Jalife J, eds. *Cardiac Electrophysiology. From Cell to Bedside*. Philadelphia: WB Saunders Company, 1995:305-313.
 9. Vassalle M, Yu H, Cohen IS. Pacemaker channels and cardiac automaticity. In: Zipes DP, Jalife J, eds. *Cardiac Electrophysiology. From Cell to Bedside*. Philadelphia: WB Saunders Company, 1999:94-103.
 10. Zhang H, Vassalle M. Role of I_{K} and I_{f} in the pacemaker mechanisms of sino-atrial node myocytes. *Can J Physiol Pharmacol* 2001;79:963-976.
 11. Anumonwo JM, Freeman LC, Kwok WM, Kass RS. Delayed rectification in single cells isolated from guinea pig sinoatrial node. *Am J Physiol* 1992;262:H921-H925.
 12. Brown H, Giles WR, Noble SJ. Membrane current underlying rhythmic activity in frog sinus venosus. *J Physiol (London)* 1977;271:783-816.
 13. Sohn HG, Vassalle M. Cesium effects on dual pacemaker mechanisms in guinea pig sinoatrial node. *J Mol Cell Cardiol* 1995;27:563-577.
 14. Liu YM, Yu H, Li CZ, Cohen IS, Vassalle M. Cs^{+} effects on i_{f} and i_{K} in rabbit sinoatrial node myocytes: implications for SA node automaticity. *J Cardiovasc Pharmacol* 1998;32:783-790.
 15. Noma A. Mechanisms underlying cessation of rabbit sinoatrial node pacemaker activity in high potassium solutions. *Jap J Physiol* 1976;26:619-630.
 16. DiFrancesco D. A new interpretation of the pacemaker current in calf Purkinje fibres. *J Physiol (London)* 1981;314:359-376.
 17. Glitsch HG, Grabowski W, Thielen J. Activation of the electrogenic sodium pump in guinea-pig atria by external potassium ions. *J Physiol (Lond)* 1978;276:515-524.
 18. DiFrancesco D, Mangoni M, Maccaferri G. The pacemaker current in cardiac cells. In: Zipes DP, Jalife J, eds. *Cardiac Electrophysiology. From Cell to Bedside*. Philadelphia: WB Saunders Company, 1995:96-103.
 19. DiFrancesco D. Block and activation of the pacemaker channel in calf Purkinje fibres: effects of potassium, caesium and rubidium. *J Physiol (Lond)* 1982;329:485-507.
 20. Kim EM, Choy H, Vassalle M. Mechanisms of suppression and initiation of pacemaker activity in guinea pig sino-atrial node superfused in high $[K^{+}]_{o}$. *J Mol Cell Cardiol* 1997;29:1433-1445.
 21. Choy Y, Kim EM, Vassalle M. Overdrive excitation in the guinea pig sino-atrial node superfused in high $[K^{+}]_{o}$. *J Biomed Sci* 1997;4:179-191.
 22. Zhang H, Vassalle M. Role of dual pacemaker mechanisms in sino-atrial node discharge. *J Biomed Sci* 2000;7:100-113.
 23. Vassalle M. An analysis of cardiac pacemaker potential by means of a "voltage clamp" technique. *Am J Physiol* 1966;210:1335-1341.
 24. Noble D, Tsien RW. The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J Physiol (London)* 1968;195:185-214.
 25. Peper K, Trautwein W. A note on the pacemaker current in Purkinje fibres. *Pflügers Arch* 1969;309:356-361.
 26. DiFrancesco D, Noble D. A model of cardiac electrical activity incorporating ionic pumps and concentration changes. *Philos Transact Royal Soc (B)* 1985;307:353-398.
 27. Vassalle M. Cardiac pacemaker potentials at different extra- and intracellular K concentrations. *Am J Physiol* 1965;208:770-775.
 28. Vassalle M, Kotake H, Lin CI. Pacemaker current, membrane resistance, and K^{+} in sheep cardiac Purkinje fibres. *Cardiovasc Res* 1992;26:383-391.
 29. Valenzuela F, Vassalle M. On the mechanism of barium induced diastolic depolarization in isolated ventricular myocytes. *Cardiovasc Res* 1989;23:390-399.
 30. Shen JB, Vassalle M. Cesium abolishes the barium-induced pacemaker potential and current in guinea pig ventricular myocytes. *J Cardiovasc Electrophysiol* 1994;5:1031-1044.
 31. Yu H, Chang F, Cohen IS. Pacemaker current exists in ventricular myocytes. *Circ Res* 1993;72:232-236.
 32. Carmeliet E. Decrease of K efflux and influx by external Cs^{+} ions in cardiac Purkinje and muscle cells. *Pflügers Arch* 1980;383:143-150.
 33. Iacono G, Vassalle M. The interrelationship of cesium, intracellular sodium activity, and pacemaker potential in cardiac Purkinje fibers. *Can J Physiol Pharmacol*

- 1990;68:1236-1246.
34. Vassalle M, Tamargo J. An analysis of calcium effects on diastolic depolarization in sheep cardiac Purkinje fibers. *J Physiol (Paris)* 1991;85:27-37.
 35. Sternlicht JP, Vassalle M. Cesium, Na⁺-K⁺ pump and pacemaker potential in cardiac Purkinje fibers. *J Biomed Sci* 1995;2:366-378.
 36. Chae SW, Wang DY, Gong QY, Lee CO. Effect of norepinephrine on Na⁺-K⁺ pump and Na⁺ influx in sheep cardiac Purkinje fibers. *Am J Physiol* 1990;258:C713-C722.
 37. Glitsch HG, Pusch H, Verdonck F. The contribution of Na and K ions to the pacemaker current in sheep cardiac Purkinje fibres. *Pflügers Arch* 1986;406:464-471.
 38. Eisner DA, Lederer WJ. Characterization of the electrogenic sodium pump in cardiac Purkinje fibres. *J Physiol (Lond)* 1980;303:441-474.
 39. Cordeiro JM, Spitzer KW, Giles WR, Ershler PE, Cannell MB, Bridge JH. Location of the initiation site of calcium transients and sparks in rabbit heart Purkinje cells. *J Physiol (London)* 2001;531:301-314.
 40. Vassalle M, Yu H, Cohen IS. The pacemaker current in cardiac Purkinje myocytes. *J Gen Physiol* 1995;106:559-578.
 41. Nett MP, Vassalle M. Obligatory role of diastolic voltage oscillations in sino-atrial node discharge. *J Mol Cell Cardiol* 2003, in press.
 42. Huser J, Blatter LA, Lipsius SL. Intracellular Ca²⁺ release contributes to automaticity in cat atrial pacemaker cells. *J Physiol* 2000;524:415-422.
 43. Bogdanov KY, Vinogradova TM, Lakatta EG. Sinoatrial nodal cell ryanodine receptor and Na⁺-Ca²⁺ exchanger: molecular partners in pacemaker regulation. *Circ Res* 2001;88:1254-1258.
 44. Vassalle M. Overdrive suppression and overdrive excitation. In: Rosen MR, Janse MJ, Wit AL, eds. *Cardiac Electrophysiology: A Textbook*. Mount Kisco, NY: Futura Publishing Co., Inc., 1990:1-15 (Section 2.2).
 45. Meissner G. Ryanodine activation and inhibition of the Ca²⁺ release channels on sarcoplasmic reticulum. *J Biol Chem* 1998;453:199-207.
 46. Spiegler P, Vassalle M. Role of voltage oscillations in the automaticity of sheep cardiac Purkinje fibers. *Can J Physiol Pharmacol* 1995;73:1165-1180.
 47. Berg DE, Vassalle M. Oscillatory zones and their role in normal and abnormal sheep Purkinje fiber automaticity. *J Biomed Sci* 2000;7:364-379.
 48. Vassalle M. Generation and conduction of impulses in the heart under physiological and pathological conditions. *Pharmac Ther [B]* 1977;3:1-39.
 49. Vassalle M. Physiological basis of normal and abnormal automaticity. In: Rosenbaum MB, Elizari MV, eds. *Frontiers of Cardiac Electrophysiology*. Boston: Martinus Nijhoff Publishers, 1983:120-143.
 50. Ferrier GR. Digitalis arrhythmias: role of oscillatory afterpotentials. *Progr Cardiovasc Dis* 1977;19:459-474.
 51. Vassalle M, Mugelli A. An oscillatory current in sheep cardiac Purkinje fibers. *Circ Res* 1981;48:618-631.
 52. Rota M, Vassalle M. Patch-clamp analysis in canine cardiac Purkinje cells of a novel sodium component in the pacemaker range. *J Physiol (London)* 2003;548.1:147-165.
 53. Lin CI, Vassalle M. Role of sodium in strophanthidin toxicity of Purkinje fibers. *Am J Physiol* 1978;234:H477-H486.
 54. Vassalle M, Lin CI. Effect of calcium on strophanthidin-induced electrical and mechanical toxicity in cardiac Purkinje fibers. *Am J Physiol* 1979;236:H689-H697.
 55. Lin CI, Vassalle M. Calcium overload and strophanthidin-induced mechanical toxicity in cardiac Purkinje fibers. *Can J Physiol Pharmacol* 1983;61:1329-1339.
 56. Kotake H, Vassalle M. Rate-force relationship and calcium overload in canine Purkinje fibers. *J Mol Cell Cardiol* 1986;18:1047-1066.
 57. Abete P, Vassalle M. Relation among Na⁺-K⁺ pump, Na⁺ activity and force in strophanthidin inotropy in sheep cardiac Purkinje fibres. *J Physiol (London)* 1988;404:275-299.
 58. Hasegawa J, Vassalle M. Enhancement and suppression of currents related to calcium-overload by different concentrations of methylxanthines. *Archives Internat Pharmacodyn Therap* 1986;282:68-81.
 59. Di Gennaro M, Carbonin P, Vassalle M. On the mechanism by which caffeine abolishes the fast rhythms induced cardiotoxic steroids. *J Mol Cell Cardiol* 1984;16:851-862.
 60. Masson-Pévet M, Bleeker WK, Mackaay AJ, Gros D, Bouman LN. Ultrastructural and functional aspects of the rabbit sinoatrial node. In: Bonke FIM, ed. *The Sinus Node: Structure, Function and Clinical Relevance*. The Hague: Martinus Nijhoff Publisher, 1978:195-211.
 61. Graziani AT, Vassalle M. Overdrive suppression and overdrive excitation in guinea pig sino-atrial node. *FASEB J* 2003;17:A116.