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Funny current provides a relatively modest contribution to spontaneous beating rate regulation of human and rabbit sinoatrial node cells

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Keywords

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In their letter to the Editor (the Letter [1]) Verkerk and Wilders utilized numerical modeling to ascertain the relative importance of the funny current (I_f) in human versus rabbit sinoatrial node. The main thrust of the model simulations in the Letter is that in both species I_f during DD is close to I_{net} defined as

$$I_{net} = -C_m dV_m / dt$$

However, we believe that the authors' approach does not demonstrate the importance of I_f for DD **in either species**, because I_{net} is determined not by a particular inward current, such as I_f , but by the sum of all inward (negative) currents and outward (positive) currents (see below).

With respect to simulation of I_f , only a single set of parameters was employed by Verkerk and Wilders. While I_f halfmaximal activation voltage ($V_{1/2}$) available in the literature for rabbit SANC greatly varies in rabbit SANC (Fig.1A), simulations of the Verkerk and Wilders Letter used the most "leftward shifted" I_f activation curve ($V_{1/2} = -76$ mV), thereby converging the I_f activation curves of the two species, as $V_{1/2}$ in humans is -97 mV (Fig.1A).

I_f activation kinetics are crucial for numerical simulations of I_f . The substantially non-linear I_f activation curve of Verkerk and Wilders, in the range of physiologically relevant membrane potentials from -100 mV to $+50$ mV, was fit using only a single data point at -40 mV (black box in Fig.1B). Accurate assessment of I_f activation kinetics in human SANC requires more data points at various potentials, especially within the DD range.

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$I_{net}=0$ at the Maximum Diastolic Potential (MDP) due to equilibration of all inward and outward currents. Following the MDP the current or conductance of a pacemaker mechanism must change in order to effect changes in this balance of ion currents, and therefore to drive the DD. Because the AP repolarization time is much longer in human vs. rabbit (Fig.1, B vs. A of the Letter), I_f is strongly (more than half maximally) activated during AP repolarization, i.e. prior to achievement of the MDP. This indicates to us, that a major role of I_f in human SANC is to prevent excessive hyperpolarization during AP repolarization, thus representing an anti-bradycardic mechanism, rather than a primary rate regulatory mechanism. Our interpretation is consistent with the experimental observation of a hyperpolarization shift of the MDP when I_f is blocked by Cs^+ in human SANC resulting in a moderate bradycardic effect (Fig.2B of the Verkerk and Wilders Letter). In some patients a small bradycardic effect (a few bpm) of I_f blockade with ivabradine could be also explained by the old idea that I_f protects the SA node from a hyperpolarizing, bradycardic influence of atria (see [2], and related discussion in [3], section “Role of I_h in Cardiac Automaticity”). Indeed, functional expression of I_f was found much more pronounced in the SA node periphery than in its center (the primary pacemaker zone of SA node) [4].

Also of note, the simulations of I_f by Verkerk and Wilders in the Letter are performed for only a basal beating rate. During stress, however, humans can achieve a heart rate of more than 200 bpm. During rapid beating the human AP cycle will have a much shorter and faster DD, requiring a much greater I_{net} and providing less time for I_f activation: thus I_{net} and I_f would diverge.

Since contributions of other important currents such as I_K , I_{CaL} , I_{CaT} , I_{NCX} , etc.. are not analyzed in the Verkerk and Wilders Letter, the impression that I_f exclusively drives the I_{net} and the DD in Fig.1 of the Letter is misleading. In Fig.2A we simulate several ion currents simultaneously by using the exact shape of rabbit AP of the Verkerk and Wilders Letter in Fig1A. Our simulations reproduced the result of Verkerk and Wilders, i.e. that I_f is indeed close to I_{net} during a mid part of the DD and I_f decreases during later DD (Fig.2A). Our model simulations of other currents (Fig.2A) demonstrate, as expected, that deactivation of I_{Kr} contributes to early and mid DD, that mid DD is strongly influenced by I_{NCX} but moderately by I_{CaT} , and that the very late DD is strongly contributed by I_{NCX} and I_{CaL} . Note in Figure 2A that even at the point of maximum I_f , substantial outward currents (e.g. I_{Kr} and I_{NaK}) as well as inward currents (I_{NCX} and $I_{Na,bkg}$) contribute to I_{net} . Furthermore, I_{NCX} increases as I_f decreases. This I_{NCX} increase is induced by late diastolic Ca^{2+} elevations (predicted by our model [5]) that have been experimentally demonstrated in different laboratories [6,7] including laboratory of Verkerk and Wilders [8]. While the contributions of I_{Kr} , I_{NCX} , I_{CaT} , and I_{CaL} to DD are established pacemaker mechanisms, a direct comparison of any one current magnitude with I_{net} (the approach undertaken in the Verkerk and Wilders Letter) does not provide any insight about its real pacemaker contribution: I_{NCX} is larger than I_{net} , I_{CaL} and I_{CaT} are smaller than I_{net} , and I_{Kr} has even a different direction than I_{net} . Furthermore, the magnitude of the inward $I_{Na,bkg}$, i.e. of a non-pacemaker current (having neither a gating mechanism, nor a dynamic intracellular regulation), is larger than I_{net} . Thus our analysis indicates that comparing I_f alone to I_{net} with respect to determining the relative contribution of I_f to pacemaker activity is meaningless. In this regard the modest bradycardic effect of I_f blockade on beating rate contrasts markedly with the cessation of automaticity when Na/Ca²⁺ exchanger (NCX) is acutely, transiently blocked by rapid, transient removal of Na⁺ from the bathing fluid (Fig.2B). This result has been confirmed in guinea pig SANC [9].

It is our opinion that informative numerical analyses of cardiac pacemaker function must include (in addition to I_f , I_K , I_{CaL}) the late diastolic I_{NCX} activation (Fig.2A) caused by the late diastolic Ca^{2+} elevations [6–8,10]. Additionally, we believe that the idea that I_f is a major component of heart rate regulation in SANC may need to be abandoned. Surely I_f contributes

to heart rate regulation, but to only a modest degree compared to other mechanisms (Fig.2B). In fact, despite the complete loss of cAMP sensitivity of I_f in mice, heart rate regulation is preserved [11]. Instead of continuing to “beat the drum” for an I_f role in pacemaker rate regulation, other dominant features of I_f might be perused. For example, recent data in genetically manipulated animals suggest that the true function of I_f is likely to be rhythm stabilization at low rates, and during rate transitions, rather than rate regulation, per se [12].

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Figure 1.

Steady state activation and kinetics of activation of I_f in rabbit and humans. Data from isolated human SANC are shown as filled squares and the associated fits as solid lines. (A) Steady-state activation curve. (B) Time constant of (de)activation. The figure is reproduced from [13] with permission. Citation numbers (marked by*) in the figure are those in the original publication[13].

Figure 2.

A: Experimentally recorded APs of a single rabbit SANC (top) (numerically reproduced from Fig.1A of the Verkerk and Wilders Letter), associated net membrane current density derived from dV_m/dt (I_{net} , red line, bottom), and numerically reconstructed ion currents (bottom panel, different colors) in rabbit SANC. The currents were simulated by a most recent numerical model of rabbit SANC [5] when all formulations of the model (including Ca^{2+} cycling) were numerically entrained by voltage clamp using the AP recording in Fig.1A of the Verkerk and Wilders Letter. Maximum conductance of I_f current was increased to 0.23625 nS/pF in order to reproduce 24 pA/pF I_f current at -130 mV as in the Verkerk and Wilders Letter. The predicted diastolic increase of I_{NCX} (magenta) was caused by the late diastolic Ca^{2+} elevation in the

submembrane space produced by diastolic Ca^{2+} release from junctional SR in the model (not shown). B: Linescan image of Ca^{2+} release with superimposed simultaneous AP records during rapid, brief superfusion in the diastolic period with a solution in which Na^+ was replaced by Li^+ . Note that the maneuver blocked the subsequent AP firing by substantially retarding the DD. Red curve superimposed on the last AP preceding spritz of Na^+ free solution (in the AP trace, zoomed in right inset) is a copy of the residual membrane potential oscillation observed during the Na^+ free solution spritz. Scanned line was oriented perpendicular to long cell axis at half its depth (top inset). From [10].