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On the Mechanism of Depolarisation and Repolarisation in the Sino-Atrial Node

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Abstract

In 16 traces of spontaneous activity of sinus node pacemaker cells of various species from mouse to man, the charge change during repolarisation was between 3 and 8 times (mostly between 3 and 4 times) that recorded during the pacemaker current ("funny current", I_f). This finding is incompatible with the necessity for a steady state of ionic movement in and out of the cell - the ionic balance; there is a deficit of outward positive ion movement sufficient to complete repolarisation. It is postulated that the deficit is solved by generation of electrical energy in the form of electrons by oxidative phosphorylation of mitochondria.

Keywords: Heart; Cardiac pacemaker; Funny current; Ionic balance

Introduction

The heart can beat 3 million times or more in a human lifetime, dependent on the sinoatrial node maintaining steady states of cardiac rhythm during every level of bodily activity. The reliability of this cardiac trigger clock depends on spontaneous depolarisation of the sinus nodal cells by an inward current, called the funny current, I_f. The current is associated with channels in the sinus nodal cell membrane [1-3] that depend on the presence of the HCN_4 protein, mutations of which affect I_f [4,5]. The term "funny" is used in the absence of firm knowledge of the charged particles that carry the current, Na^+, and K^+ dependence have been claimed, but most authors in the field assume a mixed ion dependence. Ideally, one would need to measure the increase in intracellular concentration of each ion during I_f, but this has not been possible thus far. However, an experiment by Kronhaus, Spear & Neil Moore [6] showed a decrease in extracellular K^+ concentration during I_f and an equal increase during repolarisation, indicating a K^+ steady state. The importance of a steady state for ions has been illustrated by Eisner et al. [7] in the case of calcium ions (cell entry via L-type Ca^{2+} channels matched by exit via sodium/calcium exchange (Na^+/Ca^{2+} exchange, NCX)). As the heart has to beat steadily during any period of bodily activity, all ions have to be in a steady state if there is not to be ion overload or depletion of the cell. The present investigation was undertaken to clarify ion movement in and out of sinus node pacemaker cells (SANs). The hypotheses put forward to explain the mechanisms of depolarisation and repolarisation are that electron outflow participates in the diastolic pacemaker current and electron generation particpates in repolarisation of the action potential.

Methods

A search was made in the literature for electrophysiological measurement in SANs undergoing spontaneous activity. Data was extracted from the papers of [4-6,8-14]. I_f and repolarisation current were integrated to obtain a comparison of the electric charge change during If and during repolarisation.

Results

As a starting point the tracing of Kronhaus, Spear & Neil Moore [6] was copied with permission and analysed in Figure 1. The tracing of trans-membrane potential is typical for the sinoatrial node (SAN). There is a steady decrease in negative potential from the minimum of about -60mV immediately after the previous action potential to about -40mv when another action potential is triggered. This slow decrease in diastolic negative potential is attributed to an inward current of unknown mechanism, hence "funny current", I_f. These authors inserted a potassium ion (K^+)-sensitive electrode into the extracellular space, admitting that this procedure may have effected some minor trauma. Nevertheless, while this problem may have affected the quantification, the waveform of the extracellular K^+ concentration is registered, although there may be a slight phase shift with respect to the simultaneous trans-membrane potential recording. The horizontal line indicates the mean, with the area above the line equalling the area below and confirms a steady state for K^+. A decline in extracellular K^+ was shown to accompany If, suggesting a contribution of K^+ to I_f. Sodium ions (Na^+) have also been implicated in the generation of I_f. However, it is clear

that the net current in the conventional polarity is inward, which means that there is an electron outward flow in excess of any positive ion inflow. It is also shown in Figure 1 that there is a rise in extracellular K^+ during repolarisation, suggesting a contribution of K^+ outflow towards restoration of diastolic trans-membrane potential. However, in view of the fact that the greater contribution to depolarisation was that of electron outflow, the quantitative contribution of K^+ outflow, and indeed of all positive ion outflows, must be uncertain.

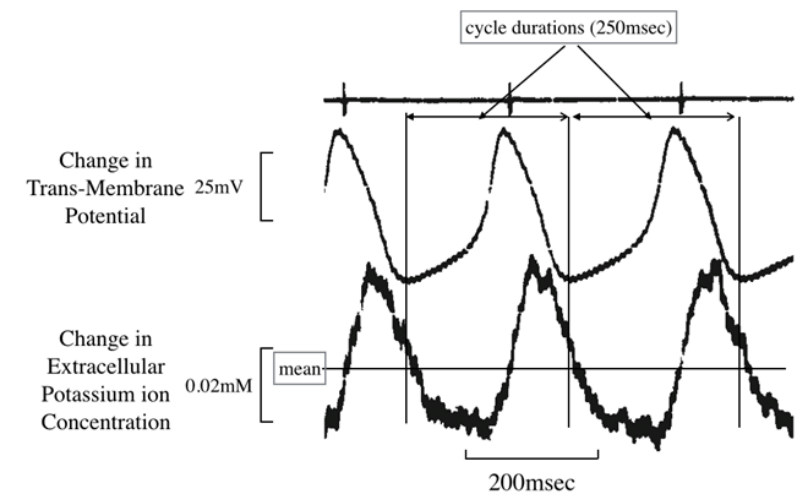

Figure 1: Simultaneous recording of SAN trans-membrane potential by Kronhaus et al. [6], shown with permission. Lines and arrows added by the author. Vertical lines indicate the start of the pacemaker current I_f. The horizontal line indicates the mean extracellular K^+ concentration. Note that extracellular K^+ declines during I_f and increases during repolarisation.

In order to clarify this uncertainty, the next step in the analysis attempted to analyse the quantitative contributions. To this end, the I_f current up to the threshold at which the upstroke of the action potential occurs was integrated and compared with the simular treatment of the repolarisation current. Conventionally the former is considered to be and inward current and the latter an outward current. The method is illustrated in Figure 2 which depicts a spontaneous SAN cycling with registration of the action potential and the trans-membrane current. The authors also measured the net current, which is tiny, in the pico-amp range, but nevertheless has the same waveform as that shown measured with the dV/dt method.

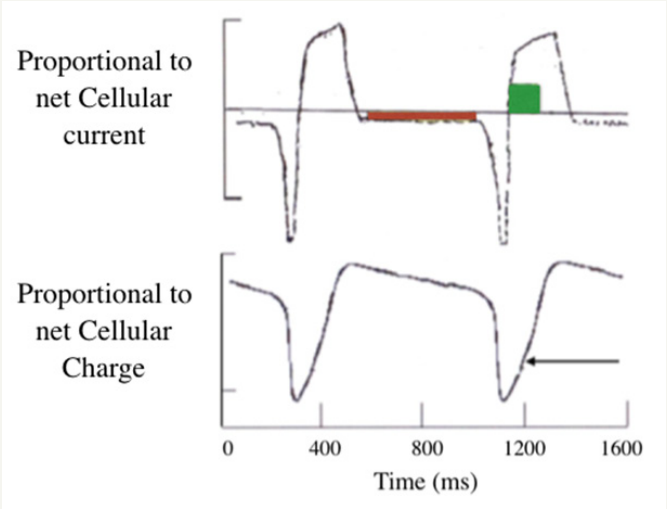

Figure 2: Spontaneous SAN cycling of a human SAN recorded by Verkerk, van Borren & Wilders [10], reproduced with permission. The lower trace shows the accompanying changes in net charge. The red rectangle denotes the maximum possible contribution (total) of positive ions to I_f. The green square has the same area indicating the maximum possible contribution of positive ion outflow to repolarisation if ionic steady state is to be maintained; this is clearly only about a quarter of the area of the repolarisation current, as also indicated by the arrow on the repolarisation rise of negative charge of the lower trace.

Net current is plotted in the downward direction indicating a net positive current inward and a net negative electron outflow, while net repolarising current is plotted upwards (positive current outward). Integration of I_f is indicated by the area coloured in red.

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The green square area under the repolarising current is equal to the area coloured red and indicates the contribution of positive charge entering with If that can contribute to repolarisation; this is of the order of 25%. The lower trace indicates the changes in net charge assuming a constant relationship between charge, being a unit of electrical energy, and potential difference, and is plotted with gain of positive charge (and loss of negative charge) downwards, and gain of negative charge (during repolarisation) upwards. The arrow during repolarisation indicates the contribution of positive charge gained during I_f.

In order to explore whether this finding is general, a total of 16 traces of spontaneous SAN activity was examined. These were published records from a number of species varying in size from mouse to human. In each case, the integrated net change in electric charge was normalised to the integrated intracellular change in positive charge during I_f. Figure 3 shows that there was a negative charge increase during repolarisation which always exceeds the intracellular increase in positive charge during I_f.

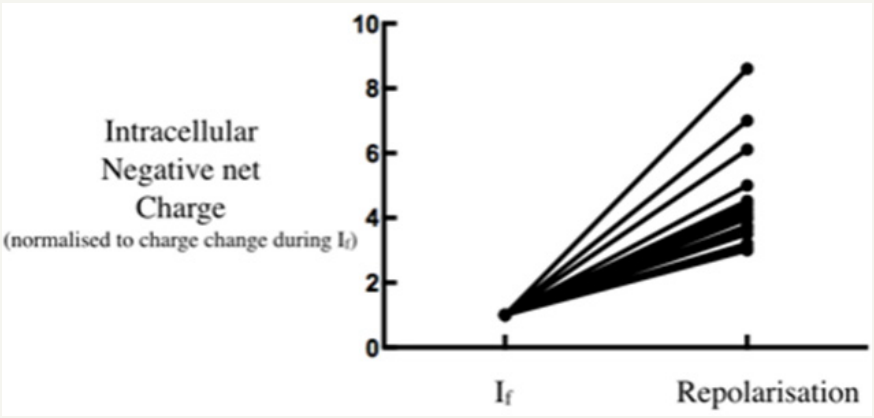

Figure 3: Gain in negative charge during repolarisation compared with positive charge gain during I_f, n=16.

Discussion

The basic definition of electricity is that the fundamental particle of electrical energy is the electron which has been assigned a negative sign. Thus, an electric current with a conventionally positive sign is achieved by a flow of electrons in the opposite direction. The observation of an inward current, the pacemaker current, I_f, during diastole in SANs is thus confirmed to be a net outflow of electrons, accompanied by a smaller inflow of positive ions, of which only K^+ seems to have been measured [6]. This analysis shows that the gain in intracellular negative charge always exceeds the net positive charge gain during I_f. That electrons flow in living tissue is established, moving from high electron density locations to lower electron density locations [15]. Electrons are produced in mitochondria during oxidative phosphorylation, which is controlled by mitochondrial membrane potential [16]. The mitochondria have a voltage of -180 to -220mV [17], so that there is a considerable gradient from this to the potential of the SAN cytoplasm at the peak of the action potential, driving electrons into the cell cytoplasm to achieve repolarisation, as the mitochondrial membrane has a finite conductance.

The obvious factor not accounted for as yet in this discussion is the Ca^{2+} gain during the action potential upstroke when the L-type calcium current is active [10]. The consequent small increase in intracellular Ca^{2+} triggers the release via the ryanodine receptors of Ca^{2+} from the sarcoplasmic reticulum to cause the majority of intracellular increase in Ca^{2+} concentration [18]. The demands of steady state necessarily determine that the Ca^{2+} gain is balanced by Ca^{2+} extrusion via NCX [7], which causes an inward current that cannot therefore contribute to repolarisation. The demands of steady state necessity also determine that the inward passage of positive ions during I_f must be balanced by an equal and opposite movement of each ion involved, of which only such K^+ movement seems to have been confirmed [6] and can be attributed to outflow of K^+ via K^+ channels. As can be seen from Figure 1, this is a rapid outflow consistent with a contribution to repolarisation; outward diffusion of K is slow [19] and would not contribute to repolarisation per beat. Confirmation of this principle of ionic balance for Na^+ is complicated by the fact that most of the intracellular Na^+ gain is by NCX, and this sets the level of Na^+ extrusion via the Na^+/K^+ ATPase sodium pump which takes about 10 minutes [20].

That the intracellular negative charge always exceeds the net positive charge gain during I_f is apparently contrary to steady state necessity, and demands a new hypothesis for its resolution. The solution put forward here relies on the basic electrical properties of the cell and is similar to that proposed for replarisation of ventricular cells [21]. In the resting state, that for a SAN corresponds to the instant before the commencement of I_f, the negatively charged particles exceed the positively charged particles, i.e., there are more atoms with extra electrons in the outer orbit than atoms with missing electrons. The latter include positively charged ions and protons, whose role in cellular function is well established and enables maintenance of intracellular physiological pH. The former include negatively charged ions and electrons. Cells convert chemical to electrical energy during oxidative phosphorylation in mitochondria. Electricity is generated, electricity being defined as electrons moving. The electrons in negatively charged ions and free

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electrons move. This fact has been denied by those who argue that this is impossible because electrons cannot move in an electrolyte solution. However the idea that the intracellular space consists of liquid has long since been disproved by magnetic resonance studies; the intracellular water is structured as in a gel, and gels can conduct electricity (electrons moving). The main argument in favour of a contribution of electrons to the electrophysiology of SAN is that the electrical path of electrons is one way, (generated intracellularly and leaving the cell to dissipate as heat). This is in contrast to the ionic system which has to be in a steady state of ion balance [22]. The mechanism of electron supply to fill the defecit of potential change from outflowing positive ions is similar to that postulated for complete repolarisation in ventricular cells [21].

Thus when the pacemaker channels open during I_f, regardless of which ion dependence is operating, electrons will move out in greater quantities than ions in, because they have one 10,000th of the mass of an ion, and therefore have 10,000 times the acceleration of ions, when an electromagnetic field is applied. Therefore it is possible that electron leakage contributes to I_f. The lost electrons dissipate as heat. In the case of repolarisation, this one way system comes into its own as further generation of electrons by oxidative phosphorylation supplies the deficit in negative charge and completes repolarisation.

Conclusion

The hypotheses put forward to explain the mechanisms of depolarisation and repolarisation, namely that electron outflow participates in the diastolic pacemaker current (I_f) and electron generation particpates in repolarisation of the action potential, could be tested. In the case of I_f, measurements are required of cationic inflow and intracellular anion losses during diastole that can account for the decline in intracellular negative charge; if they do not, one must attribute the change to a contribution of outflow of electrons. In the case of repolarisation of the action potential, which is conventionally attributed to K^+ outflow, one needs measurements of intracellular depletion of K^+, which inevitably must accompany that assumption.

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