

Cardiac cellular electrophysiology: back to basics

ESC Working Group on Cardiac Cellular Electrophysiology webinar

Tuesday 28 January 2020 from 18:00 to 19:00 CET

QUESTIONS AND ANSWERS

Answers written by Professor Edward Carmeliet

Question 1: You discussed the role of I_f in pacemaker activity. Do you have any thoughts on the relative importance of this mechanism compared to that of intracellular calcium release in generating the pacemaker?

Answer: From a pure theoretical point of view, rhythmic activity in the SAN can be reproduced by incorporating only currents present in the plasma membrane (I_f , I_{CaT} , I_{CaL} , $I_{K-deactivation}$, I_{sus}) i.e. in the absence of intracellular Ca dynamics. The question thus becomes: Do we need Ca release? Do we need a Ca clock?

Himeno et al (2011) tested this hypothesis by exposing the cell interior of a guinea pig SAN cell abruptly to a solution containing the Ca chelator BAPTA. This was done by changing from a perforated patch to a disrupted patch. Contractions ceased immediately, action potential duration and Ca^{2+} current inactivation significantly prolonged but rhythmic activity continued for minutes. A result difficult to understand in the framework of a substantial intervention of a Ca release mechanism.

In a more recent publication Torrento et al (2016) tried to answer the question whether induced or spontaneous Ca release (main point of the Lakatta hypothesis) played a role in pacemaker activity. They produced L-type Cav1.3 KO mice and found that the Ca dynamics were strongly impaired with reduction in the frequency of LCRs (local calcium release) and inhibition of Ca transients. The conclusion of this study is that the Cav1.3 channels appear to be the main trigger of LCRs seen in the late diastolic depolarization phase. LCRs should thus not be regarded as spontaneous sparks but resulting from the connection between membrane and Ca clock via the Cav1.3 channels and induced Ca release following Ca entry.

Conclusion from the two publications: the membrane clock is most important and with respect to calcium clock the induced Ca release idea wins.

Question 2: Which of your publications are you most proud of, and why?

Answer: "Adrenaline and the plateau phase of the cardiac action potential. Importance of Ca^{++} , Na^+ and K^+ conductance." Carmeliet and Vereecke, PA 1969, 313: 300-315.

Why this selection? These experiments consisted essentially of action potential measurements with microelectrodes and were simple in structure and execution. The most complicated experiments were

slope resistance measurements with two intracellular electrodes. Personally I think however that they yielded reliable and interesting data.

The action potential was dissected in two components: a fast Na spike followed by a slow Ca wave. The two components could appear and propagate independently; pure Ca-action potentials in cardiac tissue and a demonstration of slow conduction. The spike amplitude was very sensitive to Na. The secondary depolarization was dependent on Ca²⁺ concentration and could be blocked by Mn ions.

It is interesting to note that these measurements were done at a time that also the first voltage clamp data in cardiac tissue appeared. Remember however the serious critique that followed, especially in connection with attempts to measure Na and Ca current (publications by Johnson and Lieberman, 1971, *Physiol rev*, but also Noble: *The surprising heart* in JP 1984).

Question 3: What do you think is the most interesting, unanswered question in cardiac electrophysiology?

Answer: Does a 'fuzzy' space exist in cardiac tissue? (term proposed by Lederer et al 1990). Is diffusion of Na⁺ or other ions not a free diffusion but restricted.

A fuzzy space has been described for Na transported by the Na/K active pump (Bielen et al 1991), for Na⁺ as trigger for the Na-activated K⁺ channel in isolated patches (Luk personal communication), for Na⁺ influx via voltage-activated Na channels triggering reversed sodium-calcium exchange current and causing Ca²⁺ release from the SR (Leblanc and Hume, 1990).

More recently a new candidate was added (Hong et al. from the Eduardo Marban group 2014 *Nature Medicine* 2014). Growing evidence has been presented that ion diffusion in the t-tubule along the inside and outside of the sarcolemma is restricted. Cardiac t-tubules are densely folded by a particular cardiac isoform BIN1 (bridging integral), resulting in a local extracellular microenvironment that resists diffusion with the bulk extracellular space. The t-tubule becomes thus a 'slow diffusion zone' for more than one ion species.

Reports in favour did not continue however and a new contender (Lu and Hilgemann, 2017 *JGP*) has formulated serious objections against the fuzzy space hypothesis, based on experimentation and modeling. In the authors' own words: 'Na/K pump inactivation, subsarcolemmal Na measurements, and cytoplasmic ion turnover kinetics contradict the existence of Na spaces in mouse cardiac myocytes.' It is a substantial paper and will require some concentrated endeavour to absorb. So we are back to the beginnings, back to basics.

Question 4: Most drugs the patients use have an effect on QT interval and patients are always at risk for sudden death. How can we avoid these drugs?

Answer: When problems start during treatment, risk factors for drug-induced torsades de pointes should be checked: female gender, hypokalemia, hypomagnesemia, bradycardia, shortly after conversion of atrial fibrillation, congestive heart failure, left ventricular hypertrophy, high concentration of IKr blockers, presence of late Na current, congenital long QT syndrome, predisposing DNA polymorphisms.

Question 5: Is the ephaptic effect a direct mechanism in conduction tissue of the heart?

Answer: The ephaptic type of conduction is not specific for the conduction system in the heart but operative in the total heart. The promoters of this mechanism have first supported the thesis that it was an alternative mechanism for propagation of the impulse; more recently, they are in favour of a mixed-mode mechanism together with the gap junction.

Question 6: To what extent is the upstroke of an action potential altered when in a syncytium? what fraction of the Na current participates in depolarisation of down-stream cells?

Answer: I do not clearly see what you mean with the first part of the question. The whole heart is considered to act as a syncytium, although conduction of the action potential is not continuous but discontinuous. The cells are connected with each other via the gap junctions, not a simple pure cytoplasm pathway, but still allowing diffusion of ions and other small molecules.

I did not find a direct response to your second question about the fraction of the Na current. But I suppose that an answer can be given if we consider the concepts of source and sink. An action potential initiated at a site not only causes depolarization of that site (inward current) but also creates an electrical gradient between the cells and its neighbours, and thereby provides a source of current spreading to the adjacent tissue (outward current). The adjacent cells act as a current sink. The ratio of these two currents (charges) has been called the safety factor (ratio source /sink). This safety factor has been estimated to be 1.5. From this value the charge fraction that will depolarize the downstream cells can be estimated.

Question 7: Does ion accumulation/depletion in the transverse-tubular network play a role in cardiac electrophysiology under normal circumstances?

Answer: This problem was discussed in an answer to a previous question about fuzzy space. Please find part of the text of this answer.

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Question 8: Why does the action potential in some mammals have a large phase 2 phase and other mammals minimal phase 2, what is implications for L-type Ca current activation?

Answer: I can only make some remarks in the margin of how these changes appeared during evolution. A large phase 2 means long plateau, a minimal phase 2 means short plateau and action potential duration. Small animals, like mice, have a high heartrate (up to 10 per second). To make this possible the action potential should be kept short, to allow for enough filling time. Even when the systolic volume in the mice is smaller than in the dog heart, the high rate in the mouse is compensatory for the required oxygen transport. Implications for the Ca²⁺current are minimal. The Ca²⁺ current is maximal at the beginning of the action potential: Ca²⁺ current is a fast current and mice have a well-developed sarcoplasmic reticulum.

Question 9: What is your opinion about the current shift from HEK/CHO to hiPSC cells for cellular EP experiments?

Answer: Every preparation has its specific applications. The shift from HEK/CHO to hiPSC is understandable. I do not see that the HEK/CHO will disappear. But the hiPSCs have broader possibilities. The techniques have become available to generate stem cells from somatic cells or the reverse. The present developments goes even beyond the hiPSC level.

Of special importance is the development of methods to generate a biological pacemaker by the use of transcription factors such as TBX18. By local injection it is now possible to transform native cells into pacemaker cells with a minimally invasive treatment. Within a few days ventricular cells were transformed into pacemaker cells. TBX18 induced membrane and calcium clock automatism, sensitive to autonomic regulation (Kapoor et al, 2013).

Question 10: Are there any evidence about circadian rhythms in cellular electrophysiology mechanisms?

Answer: I have no knowledge of this type of property at the level of electrophysiology. The existence of circadian clocks in this field is more than probable.

Question 11: You talked about gap junctions, what is the role of the so-called 'neuronal' sodium channels in propagation of the action potential?

Answer: In order to answer this question, I have to define what is meant by 'neuronal' sodium channels.

Nine isoforms of voltage-activated sodium channels have been described. All can be blocked by TTX (tetrodotoxin). According to the sensitivity of block by TTX two groups are distinguished:

The molecules with a high sensitivity to block (K_m 10 nM) have been called 'neuronal' Na channels and are expressed in six isoforms (1.1, 1.2, 1.3, 1.4, 1.6 and 1.7.). Most are localized in the central nervous system except isoform 1.4 only present in skeletal muscle and 1.7 in the peripheral nervous system.

The remaining three 1.5, 1.8 and 1.9 are TTX-resistant (K_m 1 μ M) and have been called 'cardiac' channels. Nav1.5 is the main isoform in cardiac cells. (lateral membrane and intercalated disc). It should be stressed that neuronal channels can also be expressed in cardiac cells although. Although the expression occurs in smaller concentrations, from a functional point of view they can play an important role:

(1) In the T-tubules only neuronal channels are expressed Nav 1.1, 1.3, 1.6. and thus not Nav1.5. They are supposed to play an important role in excitation contraction coupling. Compared to Nav 1.5 their inactivation and activation voltage curves are shifted in the positive direction such that the channels remain available up to a more depolarized level. When activated they show a faster response; some have called this property a depolarization reserve. By promoting propagation of the depolarization wave to the interior of the fibre, activation of the Na channels is synchronized over the distance of the t-tubule and contraction will be improved.

(2) The same activation-inactivation characteristics of the neuronal channels will also improve conduction at the level of the intercalated disc. The relative effect will be smaller than in the excitation-contraction coupling because the main isoform is the Nav1.5.

(3) Neuronal channels have been found responsible for an important fraction of the late Na current. (Nav1.8). They are also present in the centre and periphery of the SAN (Nav1.1). Their concentration is larger in Purkinje fibres than in muscular myocytes.

Question 12: In disease where propagation is slowed such as ageing do you believe ephaptic and gap junction propagation are equally affected or is one preferentially altered?

Answer: By asking the question in this way you seem to accept that ephaptic conduction is actively participating in the propagation of the action potential. Until now, however the mechanism of ephaptic conduction is indeed a possibility but there exists no experimental evidence for its actual participation in the conduction process. I discussed this aspect in my oral presentation of the present webinar. In the section on propagation I cited A. Kléber with his conclusions: 1) no nexus, no conduction and 2) no connexins, no conduction. The nexus and connexins are essential constituents of the gap junction mechanism of propagation. For further reading I may refer to: B. J. Roth. Does ephaptic coupling contribute to propagation in cardiac tissue? Biophysical journal, 2014, 106:774-775 and E. Carmeliet. Conduction in cardiac tissue. Historical reflections. Physiological Reports, 2019, 7: e13860, 1-13 doi:10.14814/phy2.13860

Question 13: Can you discuss cellular basis of electroporation?

<p>Answer: Electroporation is a technique in which the cell membrane permeability is increased by application of an electric field to allow chemicals or DNA to be introduced in the cell. See Google Wikipedia.</p>
<p>Question 14: What ionic current is responsible for depolarisation during an early after depolarisation? Is it possible for I_{Na} to participate - or is it entirely I_{Ca}</p>
<p>Answer: The main mechanism of early afterdepolarisations is a regenerative increase in Ca^{2+} current during the slow phase of the plateau. In the narrow voltage range where activation and inactivation overlap Ca^{2+} channels can reactivate and lead to a new depolarization. Other currents however such as slowly inactivating Na^{+} (1.5 isoform channel), late Na^{+} current (1.8 isoform), NCX current, drug-induced decrease in I_{Kr} or I_{Ks}, delay the final repolarization and act as a conditioning process for reactivation of the Ca^{2+} current.</p>
<p>Question 15: Do you have any comment on the rising trend of computational modelling in cardiac cellular EP?</p>
<p>Answer: It is the aim of every scientist to express the results of his experimental measurements in a mathematical form. This was already the case with Bernstein when he used the equilibrium equation of Nernst for K ions to explain the resting membrane potential. When Hodgkin and Huxley formulated their membrane theory for the genesis of the action potential, they hypothesized that ion flow through ionic channels was regulated by the opening and closing of voltage-activated gates. Although only two currents were involved modelling was required and started. The complexity of the present situation is much amplified: from depolarization-activation to hyperpolarized activation, ion-activated, ligand-activated, inward rectifying K channels; channels in the plasma membrane but also in sarcoplasmic reticulum, mitochondria; ion channels but also exchangers, changes in ion concentration affecting the behaviour of ion channels. The present complex situation requires modeling and use of powerful computers, but I am convinced that the best results will be obtained in those research units where a good and equilibrated representation of experimentalists and scientists using modeling are collaborating.</p>
<p>Question 16: Can long AT be due to slowed conduction rather than delayed repolarisation?</p>
<p>Answer: In most of the cardiac cells under normal physiological condition conduction velocity is determined by the sodium current during the fast upstroke of the action potential. They are called fast response cells. Conduction however can also be based on an increase in calcium conduction, the slow Ca action potentials in slow response cells. This type of action potential occurs in the normal atrioventricular node but can also occur in fast response cells under condition of high external K^{+} concentration such as present in ischemia or hypoxia. Propagation is then more than an order of magnitude slower. Action potential duration is determined by the time course of the conductance changes during the plateau: slow inactivating Na current, I_{Na} late, I_{CaT}, I_{CaL}, $INCX$, as depolarising or inward currents, and I_{to}, I_{Kr}, I_{Ks}, I_{Kur} as repolarizing or outward currents.</p>
<p>Question 17: What is your most important recommendation for young PhD-student interested in cellular electrophysiology?</p>
<p>Answer: Besides the will to work hard, of major importance is the choice of a good laboratory and a good mentor. Sufficient postdocs, a tradition of excellent publications, contacts and exchange with other laboratories.</p>

Question 18: What do you think how important intracellular calcium buffering is and where is more work required in future on this field?

Answer: The fact that 99% of the cytoplasmic calcium in cardiac myocytes is bound to buffers strongly suggest that buffering is an important mechanism. The most important buffers are troponin C, SERCA, calmodulin, the sarcolemma and myosin (the latter is a Ca-Mg buffer). Alteration of buffering power will affect the systolic Ca^{2+} transient and thus also contraction. Physiologic modulation of buffering occurs through beta-adrenergic stimulation and changes in pH. Pathologic changes of buffering have been described in atrial fibrillation. During rapid atrial pacing (model of atrial fibrillation, absence or paucity of t-tubules), propagation of the calcium transient to the center of the preparation is markedly decreased. See Smith GI and Eisner D. Calcium buffering in the heart in health and disease. Circulation, 2019, 139: 2358-2371.

Question 19: How an increase of transient calcium decreases Action potential duration in atrial cells while it decreases in ventricular cells?

Answer: The formulation of the question is a little bit confusing. I suppose however that the basic problem in this case is to explain why a given change in Ca_i transient can result in prolongation of the action potential in one preparation, but in the opposite way cause shortening in another preparation.

Examples: in canine or feline ventricular cells (preparations characterized by a long plateau) an increase in Ca_i transient causes action potential shortening, and a decrease in Ca_i transient an increase of action potential duration.

In rat ventricular myocytes (preparation characterized by a short and low plateau action potential) an increase in Ca_i transient causes action potential prolongation and a decrease in Ca_i transient the opposite effect.

In order to explain this species difference one should consider that an increase in Ca_i transient exerts two opposing effects on the action potential duration: on one hand it facilitates I_{Ca} inactivation, causing shortening of the action potential, but on the other hand it enhances the I_{NCX} , which promotes lengthening of the action potential.

The two opposing changes in action potential duration can thus be explained by if one assumes that the Ca_i -induced stimulation of I_{NCX} is more important in rat ventricular myocytes, while in canine or feline preparations Ca_i induced inactivation prevails.

References: WH duBell et al. The cytosolic calcium transient modulates the action potential of rat ventricular myocytes. J of Physiology, 1991, 436: 347- 369.

Question 20: What is the impedance of a rat heart?

Answer: Impedance is the effective resistance of an electric circuit to alternating current. It is a vector consisting of two independent scalar phenomena: resistance and reactance. Impedance measurement can be used to estimate ohmic resistance and capacity of cell membranes. See Cole and Curtis. Squid giant axon.

Question 21: Which part of the action potential represents the end of repolarisation?

Answer: Phase 3 or final repolarisation is the phase following the plateau (phase 2) and brings the membrane potential back to the resting state.

Question 22: What is causing the positive deflection shown at the early phase of sodium current I-V curve?

Answer: The positive deflection shown at the early phase of the sodium current IV curve is due to the capacity current which is outward for a depolarising voltage step, followed by a background (mainly K⁺ current) deflection when the voltage step stays below the threshold for the Na⁺ current.