Title: *Investigation of the mechanism of spontaneous automaticity in cardiac nodal tissues: does the potential „pacemaker-reserve" exist?*

Principal Investigator: *Dr. Norbert Nagy*

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1. Introduction

The normal automaticity of the heart is driven by the sinus-node generating spontaneous impulses according to the momentary requirements of the body by a complex, dynamic interaction of different ion channels. Actually the current concept regarding the mechanism of spontaneous automaticity is the so-called "coupled-clock" theory which claims close interaction of intracellular Ca^{2+} movements and surface membrane ion channels during pacemaking [1]. However, the role of NCX in this interaction has not been proved by direct pharmacological interventions. In this project the role and interactions of the NCX was elucidated in sinus-node. The main question was to investigate the existence of a putative "pacemaker reserve" that could be similar to the repolarization reserve in the ventricular cells. Repolarization reserve means that redundant operations of currents are able to limit and compensate for the effect of a channel inhibition. Therefore, when 1 current is inhibited, excessive action potential lengthening could not happen. Such a reserve mechanism could provide a stable and safe pacemaker mechanism.

2. Results

Coupled function of NCX and I_f form a "pacemaker reserve" in the diastolic depolarization

The possible cooperation between the NCX and I_f was investigated in spontaneously beating rabbit right ventricular tissue. The individual application of selective NCX and I_f inhibition caused marginal and moderate (~8% and ~20% respectively) lengthening on the action potential cycle length. However, when the NCX inhibition was applied in the presence of prior I_f inhibition, the effect was increased to ~17%. Similarly, when the intracellular Ca^{2+} stores were blunted by application of ryanodine and ORM-10962 to inhibit Ca^{2+} release and NCX function, the effect of the I_f blockade was greatly enhanced (~45%). When the intracellular Ca^{2+} content was decreased by lowering the extracellular Ca^{2+} (from 1.8 to 0.9 mM), the ivabradin had increased effect on the action potential cycle length again (~50%).

These results may indicate that NCX and I_f closely cooperate in the diastolic depolarization. Inhibition of one current (I_f or NCX) may not lead to excessive action potential cycle length prolongation (20 or 8% respectively), possibly due to the compensating effect of the other current. It suggests that I_f and NCX (and possibly further currents) form a strong "*pacemaker reserve*" making the pacemaking more robust, i.e. fail-safe [2].

Reverse NCX-mediated Ca^{2+}-influx establishes a "Ca^{2+}-reserve" for pacemaking

In this experiments the possible, yet unexplored role of the reverse NCX was investigated. Theoretically, an additional Ca^{2+}-influx through the reverse NCX could contribute in the refilling of the SR Ca^{2+}-content as was suggested earlier in a numerical simulation [3]. In our experiments isolated rabbit sinus node cells were investigated where we established a normal group and a group with suppressed reverse NCX. It was found that the amplitude of the intracellular Ca^{2+} transient was smaller and the sarcoplasmic reticulum (SR) Ca^{2+} content was lower when the reverse NCX was reduced. The action potential cycle length was longer, the diastolic depolarization was slower and the APD was prolonged in the presence of blunted reverse mode. In contrast, facilitation of the reverse mode by increasing the intracellular Na^+ content shortened the action potential cycle length. However, this effect was hindered when the NCX was inhibited previously, indicating the role of the reverse NCX in the Ca^{2+} load.
These results indicate that the L-type Ca current and reverse NCX form a "*Ca^{2+} reserve*" providing more effective reloading of the sarcoplasmic reticulum. This redundant loading mechanism further increase the robustness of sinus-node pacemaking [4].

The small conductance Ca^{2+}-activated K^+ current has no role in pacemaking under normal condition

Functional small-conductance Ca^{2+}-activated K^+ current (I_{SK}) was proposed to contribute to sinus-node pacemaking mice [5]. I_{SK} could have specific importance since provides connection between intracellular Ca^{2+} changes and membrane potential alterations. In our experiments, we demonstrated the presence of I_{SK} as an apamin sensitive current, however, it had no influence on pacemaking (i.e. action potential parameters) under normal condition. Similarly, the ECG of Langendorff-perfused rabbit heart remained unaltered after apamin application.

In contrast, when isoproterenol was employed prior to apamin, the I_{SK} inhibition lengthened the action potential cycle length and the ECG R-R-interval.

These results may indicate that under normal condition the I_{SK} does not influence the sinus-node pacemaking. However, during beta-adrenergic stimulation it can provide additional repolarization that may help the sinus-node pacemaking to adapt to the higher heart rate. Therefore, the I_{SK} may make the sinus-node pacemaking more "flexible" during sympathetic stimulation [6].

Potential pharmacological benefit of NCX-I_f coupling in sinus-node

In our previous study it was demonstrated that selective NCX inhibition has no effect on electrophysiological parameters of ventricular cells [7]. Similarly, the study of Kohajda et al. [2] has shown that selective NCX inhibition only marginally prolongs the sinus-node cycle length. This marginal lengthening may be harmless, but could be potentially useful in so-called 'ventricular alternans' which are highly depends on the heart rate.

Cardiac alternans mean a regular long-short-long alteration of the action potential duration parallel with large-small-large alteration of the Ca^{2+} transient amplitude and typically occurs during high heart rate. The alternans are considered as a predictor of the ventricular fibrillation. Our study revealed that the alternans mechanism may be not related to the steepness of the restitution curve [8] suggesting that its mechanism could be rather related to the disturbances of the intracellular Ca^{2+}. In this study we found that selective NCX inhibition directly decreases both the action potential and the Ca^{2+}-transient alternans magnitude by shifting the ratio between NCX and I_{CaL} currents. Since our previous study demonstrated that NCX inhibition decreases the heart rate as well [2], the anti-alternans effect of the NCX inhibition involves both direct and indirect mechanisms that may make it to a promising future therapeutic tool against alternans development [9] however, it requires further experiments. Furthermore, computer simulations predicted that selective NCX inhibition effectively reduces alternans in heart failure as well [9].

A further important player of the alternans development could be the late sodium current (I_{NaL}) that directly influences the activity of the reverse NCX [10]. In our paper the kinetics of the I_{NaL} was characterized in human, dog and guinea-pig and revealed that dog is the best model of human in respect of I_{NaL} kinetics [11]. A recent study demonstrated that I_{NaL} inhibition

by ranolazine reduces alternans [12], however, the possible role of NCX in the effect is not known. A combined inhibition of I_{NaL} and NCX could provide novel possibilities in the pharmacological suppression of the alternans, however, this scenario requires further experiments.

The balance of I_f and I_{K1} determines automaticity in Purkinje-fibres

The Purkinje-fibres represent not only the final part of the impulse propagation system, but in emergency cases – e.g.: third-degree of atrioventricular block - could generate automaticity to serve as an escape rhythm. However, the mechanism of Purkinje pacemaking is less understood, especially in human, mainly because of the difficulties of the single cell isolation. In order to address the pacemaking mechanism of Purkinje fibres in human, a novel computation simulation model was developed based on human Purkinje fibre data from our laboratory [13] in cooperation with the Oxford Computational Science group. The simulations revealed that Purkinje-fibre pacemaking is determined by the actual balance of I_f (depolarization) and I_{K1} (repolarization). Therefore, it is feasible that in Purkinje fibre the pacemaking is also based on the interaction of currents providing the possibility of fine tuning of pacemaking [14].

Ivabradin exerted tendency of larger effects in heart failure with reduced ejection fraction patients

Our previous study indicated that the effect of ivabradin can be altered depending on the intracellular Ca^{2+} level [2]. Heart failures with preserved ejection fraction and reduced ejection fraction have marked differences regarding to the intracellular Ca^{2+} handling [15]. Since ivabradin is a frequently used drug in the treatment of both heart failure types it motivated us to investigate the effect of ivabradin in the two types of heart failure patients in a meta-analysis. The results demonstrated a tendency of higher effect of ivabradin in the case of reduced ejection fraction. These results are coherent to the findings of our previous study [2] where ivabradin exerted larger effect on action potential cycle length when intracellular Ca^{2+} was blunted [16].

Hypocalcaemia-induced sinus-bradycardia due to decreased I_{CaL} and I_{NCX} function

Patients having end-stage renal disease exert severe sinus-bradycardia before sudden cardiac death. It was hypothesized that the blood electrolyte changes could be responsible for the observed low heart rate. In order to address this issue a modified version of the Severi et al human sinus node model was used [17]. It was observed that low extracellular Ca^{2+} level directly decreases the I_{CaL} and indirectly the I_{NCX}. These changes together lead to sinus-bradycardia and could provide a feasible underlying mechanism for the sudden cardiac death in end-stage renal failure [18].

Animal models of elite exercise indicate discrepant result about sinus-node remodeling

During the grant period we did not have access to human heart failure or heart failure models. However, we had possibility to investigate 3 elite exercise animal models which condition is also associated with cardiac remodeling. These animal models were the following: swimming trained rat, and running-trained rabbit and dog models [19-22]. All models were undergoing to high intensity physical training for 3 (rat and rabbit) and 6 months (dogs) and were compared to sedentary groups. Histological, ECG and ultrasound measurements were carried out before and after the treatment, and various in-vitro measurements were carried out to delineate the potential electrical remodeling.

All models exerted marked hypertrophy and sinus-bradycardia under in-vitro conditions. In the case of rats and rabbits, the sinus-bradycardia disappeared under Langendorff-condition. This result may emphasize the critical role of increased parasympathetic tone in the rat and rabbit models. However, in dogs, the in-vitro action potential measurement from spontaneously beating right atrial tissue revealed longer cycle length and increased cycle length variability in the trained group. The underlying mechanism could be the downregulation of the I_f as was presented in previous studies, however it requires further experiments [23].

The observed discrepancy between species is not clear however, could be explained by the several electrophysiological differences between dog rabbit and rat such as distinct heart rates and ion channel expression patterns as well as differences in the action potential morphology.

Discovery and characterization of novel NCX inhibitors

During the project we developed and tested a further NCX inhibitor in cooperation with the Orion Pharma Ltd. It was found that ORM-11372 exerts even lower EC_{50} values for reverse and forward modes (5 and 6 nM respectively) without influencing the L-type Ca^{2+} current (I_{CaL}). Therefore, ORM-11372 could be a promising future experimental tool for NCX research [24].

Review papers

During the grant period 3 review papers were written. A paper exclusively addressed the role of the NCX in the sinus-node pacemaking from the earliest studies to recent results. It demonstrated how the NCX research developed and how the investigators recognized the importance of NCX in pacemaking [25]. The second review is a comprehensive work discussing almost the entire cardiac electrophysiology: characterizations of ion channels, action potential waveforms, sinus-node pacemaking, arrhythmia mechanisms, electrical alterations in cardiac diseases [26]. The third review discusses the pharmacological results as well as antiarrhythmic and positive inotropic perspectives of selective NCX inhibition [27].

3. Conclusion

The main question of this project was “Does a pacemaker-reserve exist in sinus node”? The obtained results indicate that at least 2 reserve mechanism may exist. The first one is the “*depolarization reserve*” operating during the diastolic depolarization. It could be composed by the interaction of the I_f and NCX but contribution of other currents such as the L-, and T-type Ca^{2+} currents also feasible. These interaction makes the diastolic depolarization more robust, i.e. fail-safe. The second observed reserve mechanism is the “*Ca^{2+}-reserve*”, composed by Ca^{2+}-influx through the I_{CaL} and the reverse NCX. These mechanisms provide more effective refilling of the sarcoplasmic reticulum and contribute to the robustness of pacemaking.

The interaction of currents provides safer pacemaking even if one current is inhibited or downregulated in a disease (e.g.: renal failure) or as a consequence of intensive physical training and limits the bradycardia and the increase of cycle length variability.

Furthermore, selective inhibition of the NCX could open a new highway to alternans treatment by parallel targeting the NCX both in ventricle and in the sinus-node.

4. Scientometrics of the grant period

Total full-length published papers: 17

Cumulative impact factors: 110

Number of graduated PhD-students during the project: 3

Dr. Norbert Nagy

Szeged, 28.03.2023

5. References

(The blue text colour indicates papers published associated to FK-129117 project)

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