Comparison of the Tissue Response during the Loading with Voltage-Sensitive Dye in Two Animal Models

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Abstract

Two animal models - guinea pig and rabbit - were used to record monophasic action potentials using voltage sensitive dye di-4-ANEPPS in isolated hearts perfused according to Langendorf. The hearts undergo the same isolation and loading procedures. Changes in electrogram and coronary flow are followed. During the loading and washout, prominent electrophysiological changes occur (mainly decrease of spontaneous heart rate and changes in the shape of T wave), accompanied with decrease in mean coronary flow. However, these changes differ in the two species. It may be concluded that rabbit heart is more resistant to the changes which are triggered with VSD application. Although these changes are partially reversible in guinea pig heart, this model seems to be more sensitive and thus less reliable.

1. Introduction

Voltage-sensitive dyes have been used by several research groups to record monophasic action potentials (MAPs) by optical method in a variety of heart preparations (from isolated cardiomyocytes to isolated hearts) [[1]]. This method presents sophisticated, up-to-date approach to the measurement of fine voltage changes on the membrane of cardiac cell [2]]. Simultaneous recordings of transmembrane potential by optical and microelectrode techniques have validated the high fidelity of optical MAPs compared with microelectrode recordings and demonstrated that optical MAPs detected the classic features of MAPs from various parts of the conductive system and myocardium.

2. Methods

Two animal models are employed in our laboratory to record MAPs using VSD di-4-ANEPPS [[3]] in isolated hearts perfused according to Langendorf – guinea pig and rabbit. The hearts of both species undergo the same treatment.

The experiment proceeds in the following steps: obtaining the heart, control perfusion period, loading with the dye, washout and measurement of optical MAPs under various conditions (ischemia, after pharmacological interventions, etc.).

The animal is deeply anaesthetized by xylazine and ketamine, artificially ventilated and its chest opened. Then the heart is excised with a sufficiently long segment of aorta. Next, the aorta is cannulated, the heart mounted on a Langendorf apparatus and placed in a thermostat-controlled bath (37°C) filled with the Krebs-Henseleit solution of following composition (in mM): NaCl 118, $NaHCO_3$ 24, KCl 4.2, KH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 5.5, Taurine 10 and $CaCl_2$ 1.2. The solution has to be equilibrated with 95% O_2 and 5% CO_2. The isolated heart is then perfused with the same solution at the constant perfusion pressure (90-110 mmHg) for 25 - 30 minutes – control period. All hearts exhibiting any arrhythmias during this period are excluded from the experiment.

During the whole experiment electrogram by the touch-free method is recorded and mean coronary flow monitored. Six silver-silver chloride disc electrodes (4 mm in diameter) are placed on the inner surface of the bath. ECG signals are recorded from three orthogonal bipolar leads (X, Y, and Z). The signals are amplified and digitised at a sampling rate of 500 Hz by a three-channel, 16-bit AD converter. The maximum amplitude of recorded signals varies between 100 μV and 500 μV, depending on the subject. The mean coronary flow is measured every fifth minute.

The heart is then exposed to voltage-sensitive dye di-4-ANEPPS diluted in Krebs-Henseleit solution to the concentration of 2mM (stock solution in DMF, 2μM). The tissue is perfused with this mixture for 22 - 27 minutes (according to the respective coronary flow). Again, the electrogram and mean coronary flow are monitored.

Then the period of washout follows. Its length is the same as period of loading with the dye for each respective heart. After the washout, the heart is ready for recording of optical MAPs.

Changes in spontaneous heart rate observed in all experiments were examined by measurement of RR

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intervals. RR intervals were averaged in a short period of 10 heart cycles each minute during VSD loading. Thus, physiological variations in the heart rate were suppressed.

Changes in the shape of T-waves during VSD application were studied in time domain. ECG signals were segmented by standard detection algorithms to extract fixed-length intervals that included T-waves. Distance between current T-wave to median T-wave of pre-loading control period was chosen as a parameter to be studied. The extracted T-waves were down-sampled by factor of 6 to reduce computational complexity. The *j*-th T-wave was represented by a vector V_j of 80 samples. The distance between *r*-th and *s*-th vector *V* is then

$$d_{rs} = \sum_{j=1}^{80} \left| V_{rj} - V_{sj} \right| .$$

3. Results

In this study, 15 guinea pig (both sexes, 360±80g) and 10 rabbit hearts (both sexes, 3.1±0.4kg) were included. As stated above, mean coronary flow (in ml/min) and electrogram (touch-less method) were recorded during control, loading and washout periods.

In guinea pig hearts, mean coronary flow does not change during the first half of loading, however then decreases and this decrease is present also during the wash-out period (Figure 1). In rabbit hearts, these changes of coronary flow have not been found (Figure 2).

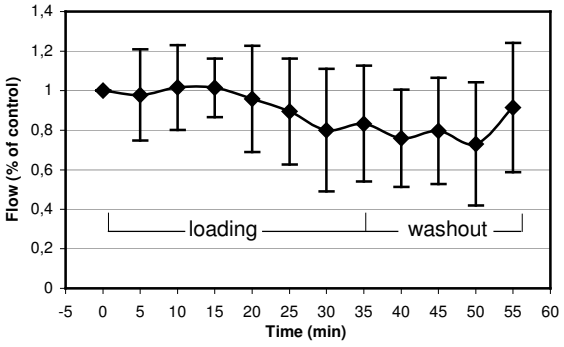

Figure 1: Mean coronary flow in guinea pig hearts during loading and washout period (0 min – flow at the end of control, 5 – 30 min loading, 35 – 55 min washout).

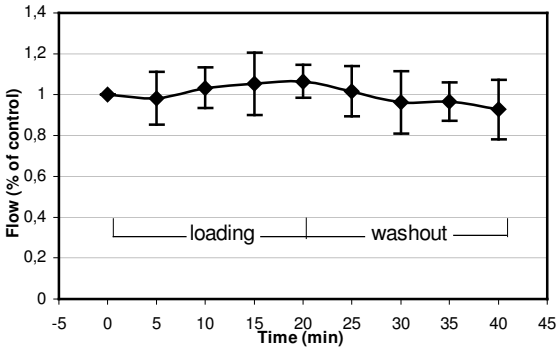

Figure 2: Mean coronary flow in rabbit hearts during loading and washout period (0 min – flow at the end of control, 5 – 20 min loading, 25 – 40 min washout).

In electrograms, during loading marked decrease in the spontaneous heart rate is present in guinea pig hearts (Figure 3), which is not observed in rabbit hearts (Figure 4). The frequency is only partially restored during wash-out period. This finding is in agreement with our previous observations [4]].

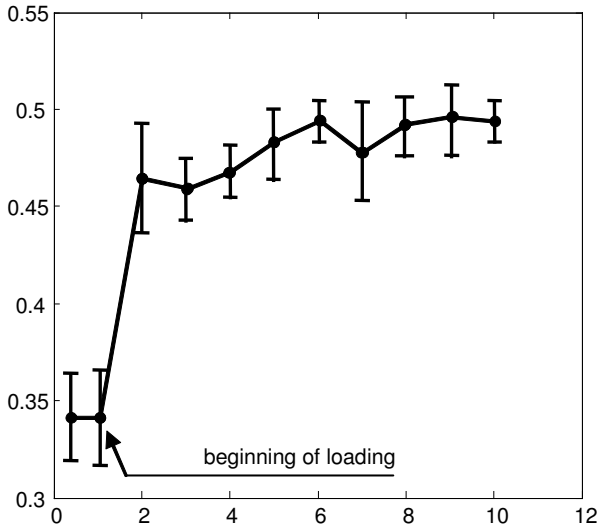

Figure 3: Spontaneous heart rate of guinea pig hearts during loading. Significant decrease can be observed 20 seconds after beginning of loading.

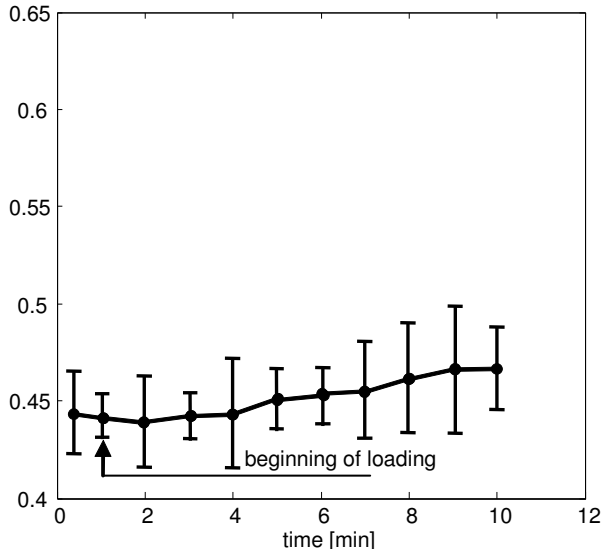

Figure 4: Spontaneous heart rate of rabbit hearts during loading.

Changes in the shape of T wave together with atrio-ventricular dissociation are other prominent findings in guinea pig model (Figure 5). These changes are only slightly indicated in rabbit hearts.

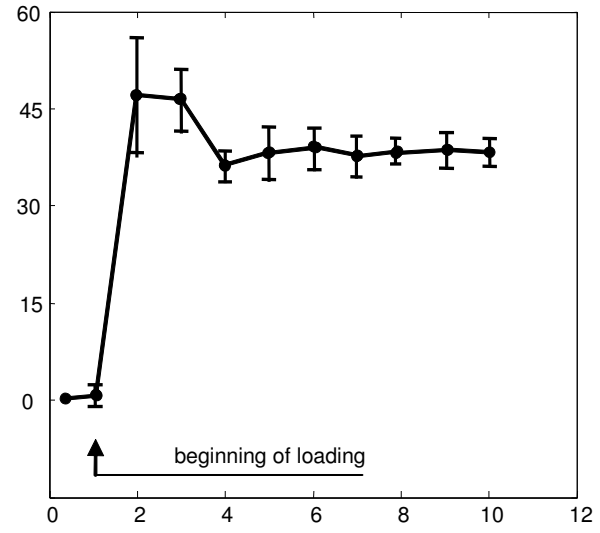

Figure 5: Distance between T-waves of guinea pig heart ECGs during loading. Significant decrease can be observed 20 seconds after beginning of loading.

4. Discussion and conclusions

The optical method of recording transmembrane action potentials has undergone a lot of improvement recently. It has approached comparable signal-to-noise ratio and thus it may spread more widely among experimental and clinical cardiology laboratories. It is generally accepted that voltage-sensitive dyes provide a powerful new tool for measurement of membrane potential in such systems where (because of scale, topology, or complexity) the use of classical electrodes is inconvenient or even impossible [[1]]. Such a situation is mainly the action potential recording in the presence of external electric fields – e.g. uninterrupted and artefact-free recording during pacing stimuli and defibrillation shocks or recording of high-resolution maps of cardiac repolarization.

The possibility to record dynamic changes in the transmembrane potential of excitable cells by optical means was first suggested in 1968 [[2]]. The first cardiac application was then introduced in 1981 – the localization of pacemaker activity in embryonic heart preparation. As stated above, the original method has been improved markedly. Also, many new voltage-sensitive dyes from various chemical groups have been tested. However, before voltage-sensitive dyes could be introduced to everyday laboratory practice, several problems have to be solved first. Firstly, it was necessary to minimize side effects of the dye on the preparation in the absence and in the presence of light, since the most prominent pharmacological effect of voltage-sensitive dyes on cardiac tissue is so-called photodynamic (or phototoxic) damage. The exact mechanism of this phenomenon remains unknown. One possible explanation is a formation of free radicals, another - direct interaction with voltage-gated calcium and/or potassium channels, which may alter the conductivity and time-dependent gating of them.

Our current results support the second hypothesis – the direct effect of voltage-sensitive dye di-4-ANEPPS on conductive system and working myocardium of guinea pig heart [5]]. In guinea pig heart, sharp slow-down of heart rate might be caused by a block of excitation propagation from the sinoatrial node to the atrioventricular node. In such case, atrioventricular node undertakes the role of a pacemaker, but of course elicits the impulses with lower frequency. Another possible explanation of this marked decrease in the heart rate in guinea pig heart is a partial block in AV node. The shape changes of electrogram support the idea of direct effect of the dye on cardiac ionic channels. Since T wave is often affected in its shape and amplitude during the loading and washout periods, we assume that predominantly potassium channels are involved in this process.

Decrease of mean coronary flow observed in guinea pig heart during loading but more markedly during reperfusion period outlasted in various extend till the end of experiment. The explanation of this phenomenon is somewhat problematic. It seems that application of

voltage-sensitive dye di-4-ANEPPS causes vasoconstriction in coronary system in isolated guinea pig heart perfused according to Langendorf. It is disputable whether there are any functional or morphological consequences of this decrease in coronary flow. According to our unpublished observations, there are no relevant changes in contractility and/or morphology in the guinea pig cardiac muscle treated with VSD di-4-ANEPPS.

What is necessary to stress, in rabbit heart only minor changes in mean coronary flow can be observed. Also prolongation of R-R intervals and changes in the morphology of T wave are small and insignificant, especially when compared with the marked changes in all these followed parameters in guinea pig hearts.

The problem with affected coronary flow can be solved in two ways: either to choose another experimental model (as rabbit heart in our case) or to keep coronary flow constant by a pump [[6], [7]].

Thus, it may be concluded that rabbit heart is more resistant to the changes which are triggered by VSD application. Although these changes are partially reversible in guinea pig heart, this model seems to be more sensitive and thus less reliable, especially when concerning studies of conductive system.

Acknowledgements

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All experiments followed the guidelines for animal treatment approved by local authorities.

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