Electrophysiological effects of Voltage-Sensitive Dye di-4-ANEPPS in Rabbit and Guinea Pig Isolated Heart

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Abstract. Voltage-sensitive dye (VSD) di-4-ANEPPS is currently used in our laboratory for recording of monophasic action potentials (MAPs) in isolated hearts. In this study, its electrophysiological effects on rabbit and guinea pig myocardium were compared during staining with VSD and washout from it. Electrograms were recorded and heart rate changes and the types and frequency of arrhythmias were evaluated. Althought certain electrophysiological changes were present in the hearts of both species, these changes are mostly reversible, especially in rabbit hearts. Thus, rabbit myocardium can be considered a reliable model for electrophysiological studies, especially when generation and propagation of the impulse is studied. It may be concluded that rabbit isolated heart is more suitable model for electrophysiological studies in the isolated heart model than guinea pig heart.

1 Introduction

Although the classical suction electrodes are still considered golden standard for recording of monophasic action potentials, there are various new methods appearing in basic cardiology laboratories enabling the researchers to record the electrical changes on the membrane of one cardiomyocyte or from small area on the surface of the heart. One of them is mapping of cardiac electrical activity employing the voltage-sensitive dyes (VSDs). After a considerable effort to improve this method it is now considered as a valuable tool for electrophysiological studies focused on numerous, most often studied topics in cardiovascular system physiology and pathophysiology, such as ischemia, reperfusion, arrhythmias generation, preconditioning, postconditioning, etc.

VSDs undergo changes in their electronic structure, and consequently their fluorescence spectra and thus respond to changes in the surrounding electric field. Therefore, they may be used successfully for instance for recording of monophasic action potentials (MAPs) in various heart models. It is very important to choose a proper kind of VSD, since staining of the heart tissue with the dye should not result in pharmacological or toxic effects. From all groups of VSDs which are available at present in the market, VSD di-4-ANEPPS is considered to be the most appropriate for myocardial tissue. It is currently used in our laboratory for recording of MAPs in the well established model of isolated heart.

This paper summarises continuation of our previous, pilot study which was focused on evaluation of electrophysiological effects of the abovementioned dye on rabbit and guinea pig isolated hearts. In the present paper, touch-less electrograms - recorded during staining with the dye and during washout from the excess of it - were examined and compared. Beside the heart rate changes, the incidence of various types of arrhythmias and their frequency was evaluated.

2 Methods

Five New Zealand rabbits (average body mass 2.89 ± 0.20kg) and three guinea pigs of non-specified breed (average body mass 403.33 ± 41.10g) of both sexes were included in this

study. The rabbits were pre-medicated with Apaurin (diazepam, 2mg i.m.) and then anaesthetised by intramuscularly applied mixture of Rometar (xylazin, 2mg/kg) and Narcamon (ketamin, 60mg/kg). First, the rabbit was artificially ventilated through tracheal cannula and then the chest was opened and the heart excised with sufficiently long piece of the aorta.

The guinea pigs were deeply anaesthetised by inhalation of ether. After subsequent cervical dislocation, the chest was quickly opened and the heart excised.

After the isolation, the hearts from both species were perfused with Krebs-Henseleit (K-H) 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 was continuously oxygenated with mixture of 95% O_2 and 5% CO_2. The perfusion was performed on Langendorff apparatus modified previously in our laboratory [1]. The mode of perfusion with constant perfusion pressure (85mmHg) was chosen. All experiments were performed at 37°C. During the whole experiment, the heart was placed in thermostat-controlled bath (37°C) filled with Krebs-Henseleit solution of abovementioned composition.

Spontaneously beating hearts were allowed to stabilize for 20-30 minutes. All preparations exhibiting any arrhythmias during the stabilization were discarded. After this control period, the tissue was stained with di-4-ANEPPS (stock solution in DMSO to final concentration of 2μM) which was diluted in K-H solution (up to concentration of 2mM) and applied into the perfusion set-up and via coronary arteries into the heart. This period lasted 20-25min on average. Subsequently, the isolated hearts were perfused with dye-free K-H solution for another 20-25min. This period was marked as washout. At this point, the tissue was ready for measurement of MAPs by optical probe.

The measuring device has been described previously [2]. Briefly, the optical system consists of a flexible bifurcated fibre cable with seven optical fibres (six illumination fibres positioned in a circle and a detection fibre positioned in the centre of the cable). The fibre optics together with micromanipulator in the bath of perfusion system enables the user to scan action potentials from various places on the heart surface with almost no mechanical constraint. The optical probe is softly attached to the preparation to suppress motion artefacts without a need of focusing. The motion artefacts are diminished by slight restriction of the preparation by plastic circle placed around the heart.

The "input" end of the cable with six illumination fibres is connected to a light source. The "output" (detection) fibre is connected to a light detector that senses the beam of emitted light. The optical fibres are protected by a silicon inner tube and a flexible chrome plated brass outer tubing. The tubing also gives stress relieve.

The cold light source with high intensity light output is used for excitation of the dye (150W halogen). It contains a built-in IR filter which prevents a preparation from heating, and a band-pass filter (560 nm +/- 30 nm), which selects light at excitation maxims of the used dye. The light intensity can be adjusted by a crescent shaped diaphragm and by controlling the lamp voltage.

The changes in dynamics of transmembrane potential result in amplitude modulation of the emitted light. This is detected by a photodiode detector with a high-pass (>610 nm) filter. The output signal of the photodiode detector is pre-amplified so that the two stage amplifier adjusts the signal to input range of data acquisition card (±1 V). The electrical circuits include also an analogue anti-aliasing filter (low-pass filter fc=2 kHz) and a high-pass filter (fc=0.05 Hz) to suppress DC offset.

The data acquisition card processes the pre-amplified and filtered signal. The card digitizes the signal with 12 bits dynamic range and at rate of 4000 samples/sec. The digital signal is stored on a hard disk for further off-line processing (noise suppression, visualization and analysis). Data acquisition is controlled by subroutines of a software package LabView.

During the whole experiment, electrograms are continually recorded from three orthogonal bipolar leads (X, Y, and Z) by the touch-free method [3]. Six silver-silver chloride disc electrodes (4mm in diameter) were placed on the inner surface of the bath. The electrograms were amplified by a set of three biological amplifiers DAM50 (World Precision Instruments, USA) and further simultaneously digitized by 16-bit AD converters at rate of 2000 samples/sec using a data acquisition multifunction card PCI-6250 (National Instruments, USA). The acquisition card also provides pre-amplification of the signals and their filtering by anti-aliasing filters. The digital signals are stored on a hard disk for further off-line processing (noise suppression, visualization and analysis). As in the case of optical signal, data acquisition is controlled by subroutines of a software package LabView (National Instruments, USA).

The recorded electrograms were analyzed and the heart rate (HR) changes were evaluated from measured and averaged ten R-R intervals at the end of each fifths minute during both – staining and washout – intervals. The results were then normalized to the end of control (100%).

The incidence of arrhythmias was noted, especially their severity and frequency of appearance. Each examined heart was given a score from 0 to 5 according to Lambeth convention [4]. Lambeth score classifies the heart according to the most severe kind of arrhythmia appearing during the particular part of experiment (0 – no arrhythmia, 1 – single premature ventricular complexes, 2 – salvos, 3 – ventricular tachycardia, 4 – reversible ventricular fibrillation, 5 – sustained ventricular fibrillation, lasting more than 2 minutes).

3 Results

Perfusion with di-4-ANEPPS caused specific changes of electrograms in all examined hearts of both species included in this study. Numerous types of arrhythmias were observed, namely AV-blockades, single ventricular extrasystoles, and monomorphic ventricular tachycardia. However, the incidence of these types of arrhythmias exhibited marked difference in guinea pigs and in rabbits.

In guinea pig hearts, mainly disturbances of impulse generation and propagation were observed. These electrical disorders were expressed as so-called “floating” P wave (original recording see in Fig. 1) and AV-blockades of low degree (partial block in the atrioventricular node). These arrhythmias were present predominantly during the staining period. During staining and washout periods, occasional premature ventricular complexes were found in one and in two hearts, respectively. According to Lambeth convention, the guinea pig group reached score 1 during staining and score 1 during washout period.

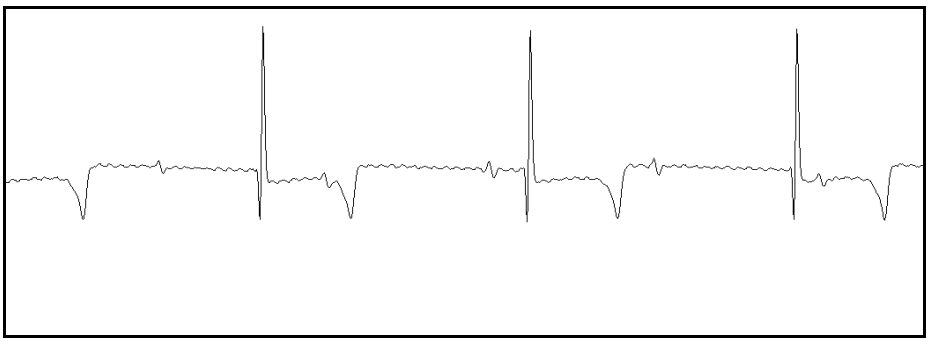

Fig 1. Original recording of 3D-electrogram in isolated guinea pig heart (the most frequent arrhythmia in this species - „floating“ P wave - during staining period)

In rabbit isolated hearts, only a few premature single ventricular complexes appeared during staining period (see Fig 2) in two hearts and only one of examined rabbit hearts showed a brief episode of monomorphic ventricular tachycardia in washout period. Otherwise, the rabbit hearts did not exhibit any rhythm disturbances. The rabbit group was assigned score 1 during staining and score 3 during washout period. When the hearts from all animals are taken in consideration - including those which did not exhibit any arrhythmias - the results reveal that there is no significant difference in arrhythmia occurrence and incidence between two tested groups (see Table 1).

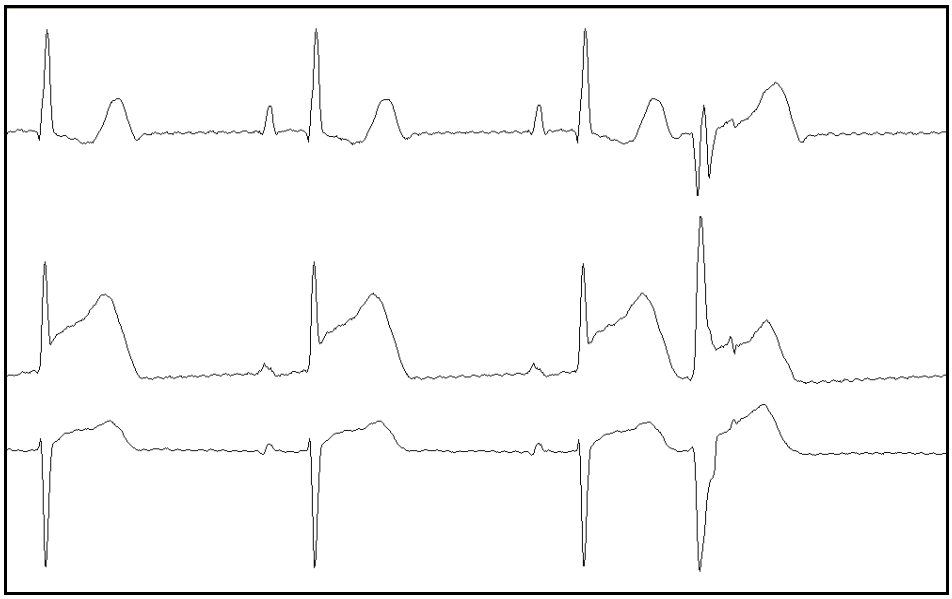

Fig 2. Original recording of 3D-electrogram in isolated rabbit heart (the most frequent arrhythmia in this species - ventricular extrasystole - during staining period; note the ischemia-like changes of electrogram, which dissappear spontaneously after couple of minutes of perfusion with VSD)

	Staining	Washout
Guinea pig 01	0	0
Guinea pig 02	1	1
Guinea pig 03	0	1
Guinea pig - average	**0.33**	**0.66**
Rabbit 01	0	0
Rabbit 02	1	0
Rabbit 03	0	0
Rabbit 04	0	0
Rabbit 05	1	3
Rabbit - average	**0.4**	**0.6**

Table 1. Summary of Lambeth score in examined hearts of both species

Normalized heart rate decreased in the isolated hearts from both species. However, there was a difference obvious - in rabbit hearts, this decrease was less steep and during the following part of experiment HR recovered (not shown); in guinea pig hearts the effect of VSD was more pronounced (see Fig. 3). When compared to the end of control (e.g. 100%),

both species showed significant decrease in the normalized heart rate ($p<0.01$, unpaired T-test, two-tailed P value). Moreover, the difference between the normalized heart rate in isolated hearts of both species was statistically significant ($p<0.01$, unpaired T-test, two-tailed P value). Thus, we can assume that the decrease in guinea pig heart rate is more significant.

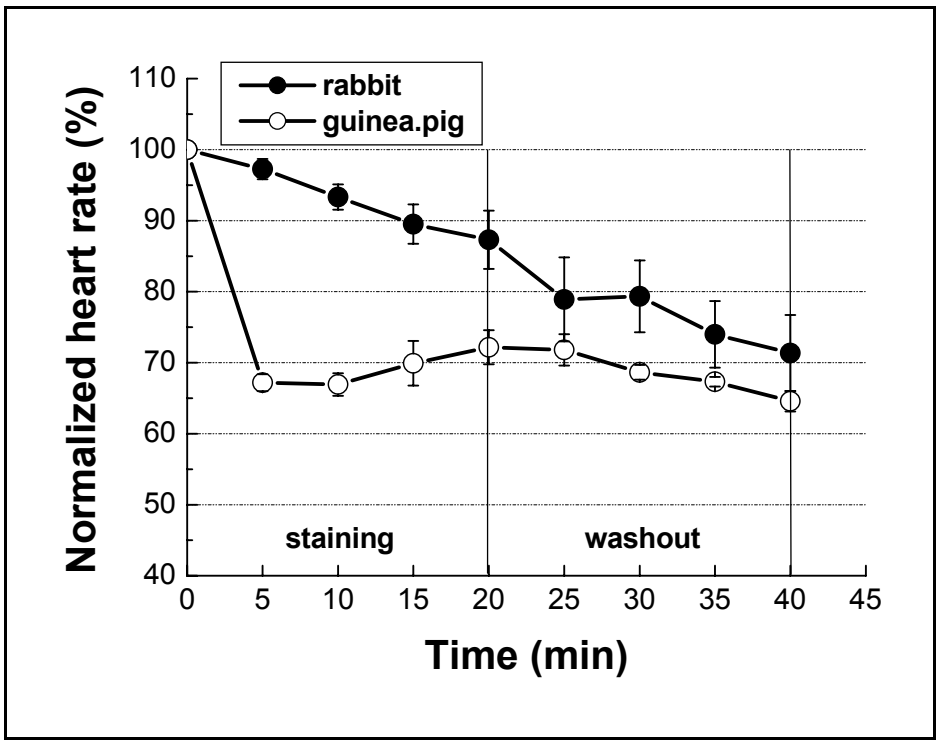

Fig 3. Normalized heart rate changes during staining and washout periods in guinea pig (open circles) and in rabbit (full circles) isolated hearts

4 Discussion

Although the possibility of recording the dynamic changes of the transmembrane potential of excitable cells by optical method was suggested already in 1968 and the first cardiac application was reported in 1981, the information about the direct response of the heart muscle to staining with VSDs is quite rare in the world literature at present. Since the introduction of this method, it has been improved markedly and numerous VSDs from various chemical groups have been tested. The researchers faced several problems with these new substances which had to be solved first, before the introduction of these dyes into everyday laboratory practice. One of the most important tasks during the preparation of heart muscle for the electrical changes measurement is the minimalization of side effects of the dye on the heart preparation. Most prominent pharmacological effect of VSDs on cardiac tissue is so-called photodynamic and phototoxic damage. Formation of free radicals or direct interaction with the voltage-gated calcium and/or potassium channels have been suggested, which may result in altered conductivity and the time-dependent gating [5, 6].

Most often used experimental models in basic cardiology research – rat, guinea pig and rabbit hearts – differ mainly in repolarization phase of action potential. The guinea pig ventricular cardiomyocytes do not develop transient outward current which – in turn – is present in rat and about half of the rabbit ventricular myocytes. Moreover, delayed rectifier current of relatively high amplitude has been found in guinea pig cardiac cells on the contrary

to negligible or even absent delayed rectifier current in rat and rabbit. Inward rectifier potassium current is similar in rabbit and guinea pig [7]. Thus, we can presume that different response of guinea pig and rabbit myocardium to staining with di-4-ANEPPS in our experiments is caused by the effect of this dye on characteristics of delayed rectifier and/or transient outward currents.

Staining of the myocardium of both examined species with VSD di-4-ANEPPS obviously leads to disturbances in the heart electrophysiological picture, expressed as a range of arrhythmias of various severity. In our experimental setup, all of these changes were mostly reversible in the case of rabbit myocardium. The only persistent change in rabbit hearts (up to certain degree) was the heart rate decrease. In guinea pig hearts, the heart rate decline was more pronounced and persistent behind the followed period in our experiments. Moreover, in this species apparent disturbances in electrical impulse generation, propagation through atria and conduction from atria to ventricles appeared. Inasmuch as this finding, we suggest that the rabbit myocardium should be considered more resistant to the electrophysiological effects of VSD di-4-ANEPPS.

5 Conclusions

We have proved that althought certain electrophysiological changes were present in the hearts of both species, these changes are mostly reversible, especially in the case of rabbit heart. Thus rabbit myocardium can be stained with di-4-ANEPPS and be considered a reliable model for electrophysiological studies, especially when generation and propagation of the impulse is studied. It may be concluded that rabbit isolated heart is more suitable model for studies at the level of electrophysiology of the whole heart.

Acknowledgement

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