Comparison of Voltage-Sensitive Dye di-4-ANNEPS Effects in Isolated Hearts of Rat, Guinea Pig, and Rabbit

Katerina Fialova[1], Jana Kolarova[2], Ivo Provaznik[2], Marie Novakova[1]

[1]Department of Physiology, Faculty of Medicine, Masaryk University Brno, Czech Republic

[2]Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Czech Republic

Abstract

Voltage-sensitive dyes (VSDs) are used for recording of monophasic action potentials (MAPs) by optical method in excitable tissues. Their direct effects on the tissue are not completely elucidated. Previously we studied effects of VSD di-4-ANEPPS during staining and washout in Langendorff-perfused guinea pig and rabbit isolated hearts. However, most often used species in basic cardiology is rat. Therefore, we decided to compare the electrophysiological effects of VSD in isolated hearts of rabbit, guinea pig, and rat.

Touch-less electrogram was recorded, the heart rate assessed and normalized. The type and incidence of arrhythmias were evaluated.

Based on our results - although the procedure of staining with the dye affects electrophysiological properties of the myocardium in all studied species - rat heart may be considered as the most suitable for electrophysiological studies using di-4-ANEPPS.

1. Introduction

Although the classical suction electrodes are still considered golden standard for recording of monophasic action potentials (MAPs), 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, frequently studied topics in cardiovascular system physiology and pathophysiology, such as ischemia, reperfusion, arrhythmias triggering, preconditioning, postconditioning, etc.

Voltage-sensitive dyes undergo changes in their electronic structure, and as a consequence also in their fluorescence spectra. These changes result from changes in the surrounding electric field, for instance in excitable tissues such as myocardium or neurons. Therefore, VSD may be used successfully for recording of MAPs in such models. Various VSDs have been introduced into everyday laboratory practice (merocyanine, ANEPPS, etc.). Dyes from ANEPPS group (amino-naphthyl-ethenyl-pyridinium) are the most constantly used in cardiac preparations [1,2]. One of them, di-4-ANEPPS is utilized in our laboratory for recording of MAPs by optical method in the well established model of isolated heart of various animal species. Most often employed experimental model in our projects is isolated rabbit heart perfused according to Langendorff.

In our previous studies, we focused on electrical changes caused by VSD di-4-ANNEPS during staining and washout in rabbit and guinea pig hearts. However, often used species in basic cardiology studies is rat. Therefore, we decided to compare the electrophysiological effects of VSD in isolated hearts of the two abovementioned species and of rats.

2. Methods

Five New Zealand rabbits (average body mass 2.89 ± 0.20 kg), three guinea pigs (average body mass 403.33 ± 41.10 g) and three Wistar rats (average body mass 257.25 ± 32.10 g) of both sexes were included in the study.

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

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

After the heart isolation, the heart was firmly fixed to

perfusion set-up by the stump of aorta and then placed in thermostat-controlled bath (37°C) filled 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 95% O_2 and 5% CO_2. The heart was then perfused with the same solution at the constant perfusion pressure (80 mmHg) for 25 - 30 minutes – control period. The perfusion was performed on Langendorff apparatus modified previously in our laboratory [3]. All hearts exhibiting any dysrrhythmias during control period were discarded. Then the tissue was stained with di-4-ANNEPS (Molecular Probes, Eugene, OR, USA) applied via coronary arteries with perfusion solution. This period lasted 20-25 min on average (depending on coronary flow). Next, the hearts were perfused with dye-free K-H solution (20-25 min, washout). After the excess of VSD was washed out, exposure to light source and recording of MAPs started.

MAPs were recorded by the optical system [4]. It consists of a flexible bifurcated fibre cable with seven optical fibres (six illuminations 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 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.

The block diagram of the acquisition system is depicted in Figure 1.

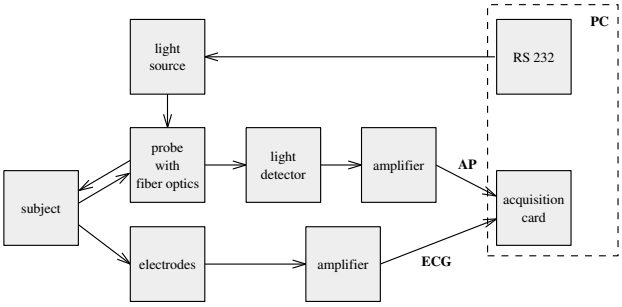

Figure 1. The block diagram of the acquisition system. The excitation light is generated by a light source Intralux DC-1100 with a 150W tungsten-halogen lamp. The light is led by flexible fiber optics to the sample. Fluorescent light is emitted by voltage-sensitive dye present in the sample and led back by the parallel fiber optics. The emitted light hits a photodiode detector. An electrical signal from the detector is amplified and digitized.

During the whole experiment, touch-less electrogram was recorded from three orthogonal bipolar leads (X, Y, and Z) [5]. Six silver-silver chloride disc electrodes (4 mm in diameter) were placed on the inner surface of the bath in which the heart was placed during the experiment. The signals were amplified by a set of three biological amplifiers DAM50 (World Precision Instruments, USA) and further simultaneously digitized by 16-bit AD converters at a sampling rate of 2000 samples/sec using a data acquisition multifunction card PCI-6250 (National Instruments, USA). The digital signals are stored on a hard disc for further off-line processing.

An example of synchronous recording of electrogram and MAPs is given in Figure 2.

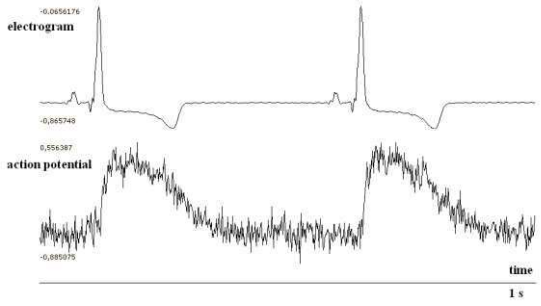

Figure 2. Synchronous recording of electrogram by touch-free method (top, one bipolar lead) and corresponding MAPs by optical method (bottom) from isolated rabbit heart perfused according to Langendorff. Recording at control conditions (37°C, spontaneously beating heart), unfiltered signal.

The recorded electrograms were analysed and the heart rate (HR) changes were evaluated from manually measured and averaged ten R-R intervals at the end of each fifths minute during both – staining and washout – periods. The results were then normalized to the end of control period (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 [6]. 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 [PVCs], 2 – salvos, 3 – ventricular tachycardia, 4 – reversible ventricular fibrillation, 5 – sustained ventricular fibrillation, lasting more than 2 minutes).

3. Results

Perfusion of the isolated hearts with VSD di-4-ANEPPS caused specific changes of electrograms in all examined species. Numerous types of arrhythmias were observed, mainly AV-blockades, single ventricular extrasystoles, and monomorphic ventricular tachycardia. However, their incidence differed markedly among the species.

In rabbit isolated hearts, only a few PVCs appeared during staining period 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.

In guinea pig hearts, mainly disturbances of impulse generation and propagation were observed. These electrical disorders were present as s.-c. AV-blockades of low degree (partial block in the atrioventricular node. These arrhythmias appeared predominantly during the staining. During staining and washout periods, occasional PVCs 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.

Only one rat heart reached score 1 during staining (i.e. PVCs were observed). During washout period, no arrhythmias were found.

When the hearts from all species 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 rabbits and guinea pigs. Rat isolated hearts exhibited only moderate electrophysiological changes (see Table 1).

	Staining	Washout
Rat 01	0	0
Rat 02	0	0
Rat 03	1	0
Rat – average	**0.33**	**0**
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 all examined hearts during staining and washout.

Normalized HR decreased in the isolated hearts of all species ($p<0.01$, unpaired t-test, two-tailed P value). However, there were obvious differences. During staining, this decrease was least steep in rabbit, then successively in rat and in guinea pig hearts. In rabbit hearts, this decrease recovered during the following part of experiment HR (not shown); in guinea pig hearts the effect of VSD was more pronounced (see Fig. 3). The highest degree of recovery during washout was observed in rat hearts.

The difference between the normalized HR in isolated hearts of each species was statistically significant ($p<0.001$, unpaired t-test, two-tailed P value). Thus, we can assume that the decrease in guinea pig HR is the most significant.

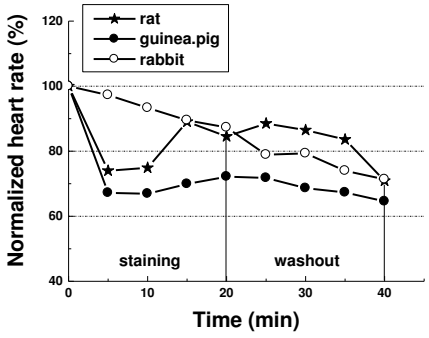

Figure 3. Normalized heart rate changes during staining and washout periods in rat (stars), guinea pig (full circles), and rabbit (open circles) isolated hearts.

4. Discussion and conclusions

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 direct response of the heart muscle to staining with VSDs is scarce 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. 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 [7].

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 [8]. Thus, we can presume that different responses of rat, guinea pig, and rabbit myocardium to staining with di-4-ANEPPS in our experiments are caused by the effect of this dye on characteristics of delayed rectifier and/or transient outward currents.

In all examined species, staining of the myocardium with di-4-ANEPPS obviously leads to disturbances in the electrophysiological picture, expressed as a range of arrhythmias of various severities. In our experimental set-up, all of these changes were mostly reversible. In rabbit hearts, the only prominent change during staining was slight HR decrease. In guinea pig hearts, the HR 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. The normalized HR of isolated rat hearts showed tendency to decrease during the staining period, but soon recovered. Although the procedure of staining with the dye affects electrophysiological properties of the myocardium in all tested species, these changes were mostly insignificant and reversible, especially in the case of rat heart. Thus, rat myocardium may be considered to be the most suitable for electrophysiological studies using di-4-ANEPPS. However, rat cardiomyocyte differs markedly from the human one. In order to advance towards clinically applicable research, the rabbit myocardium should be employed in such studies as the second choice as it was previously proved to be resistant to the changes triggered by VSD application [7].

Acknowledgements

All experiments followed the guidelines for animal treatment approved by local authorities and conformed to the EU law.

This work was supported by the grant projects of the Grant Agency GACR 102/07/1473 and MSM 0021622402.

References

[1] Loew LM. Potentiometric dyes: Imaging electrical activity of cell membrane. Pure Appl. Chem. 1996;68:1405–9.

[2] Salama G. Optical mapping: background and historical perspective. In: Rosenbaum DS. And Jalife J. Optical Mapping of Cardiac Excitation and Arrhythmias 2001. Futura Publishing Company, Inc. Chapter 1:9-31.

[3] Novakova M, Moudr J, Braveny P. A modified perfusion system for pharmacological studies in isolated hearts. In: Analysis of Biomedical Signals and Images. Biosignal 2000. Brno: Vutium Press, 2000;162-4.

[4] Provaznik I, Novakova M, Vesely Z, Blaha M, Chmelar M. Electro-optical recording system for myocardial ischemia studies in animal experiments. Computers in Cardiology 2003. Boston: IEEE, 2003;30:573−6.

[5] Uematsu T, Vozeh S, Ha HR, Follath F, Nakashima M. Method for stable measurement of the electrocardiogram in isolated guinea pig heart. J. Pharmacol. Methods 1987;18: 179–185.

[6] Walker MJA, Curtis MJ, Hearse DJ, Campbell RWF, Janse MJ, Yellon DM, et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. Cardiovasc Res 1988;22:447-455.

[7] Novakova M, Bardonova J, Provaznik I, Taborska E, Bochorakova H, Paulova H, Horky D. Effect of voltage sensitive dye di-4-ANEPPS on guinea pig nad rabbit myocardium. Gen Physiol Biophys 2008;27:45-54.

[8] Varró A, Lathrop DA, Hester SB, Nánási PP, Papp JGY. Ionic currents and action potentials in rabbit, rat, and guinea pig ventricular myocytes. Basic Res Cardiol 1993;88:93-102.

Address for correspondence.

Kateřina Fialová
Department of Physiology
Faculty of Medicine, Masaryk University
Kamenice 5
625 00 Brno
Czech Republic
katy.fialova@email.cz