Model of Preconditioning in Guinea Pig and Rabbit Isolated Hearts Loaded with Voltage-Sensitive Dye Di-4-ANEPPS

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Abstract

Cardiovascular system diseases are frequent cause of morbidity and mortality in Western countries. In our laboratory, early stages of ischemia - prominent diagnosis in this group - are examined by recording electrograms and action potentials by optical probe in isolated mammalian hearts loaded with voltage-sensitive dye (VSD) di-4-ANEPPS. In this study, guinea pig and rabbit isolated hearts were exposed to special protocol of s.-c. preconditioning - attenuation of the symptoms during repeated exposure to ischemic insult. In guinea pig myocardium, additive effect of VSD and ischemia triggered more serious and more frequent arrhythmias then the same situation in rabbit hearts. Moreover, expected attenuation of these effects was not observed, on the contrary to rabbit myocardium, where typical preconditioning was present. Rabbit myocardium appears to be more suitable for studies in such experimental design.

1. Introduction

Cardiovascular system disorders are the leading cause of mortality and morbidity in developed countries; myocardial ischemia represents very frequent diagnosis in this vast group of diseases. It is the reason why ischemia of cardiac muscle has been repeatedly investigated in experimental as well as clinical studies. There are several phenomena related to this subject, such as myocardial infarction, preconditioning, postconditioning, etc. Various models and species have been employed in studying these events.

In our laboratory, we search for tools for detection of early stages of myocardial ischemia from electrophysiological data (electrogram and monophasic action potentials recorded by optical probe using voltage-sensitive dyes) in the model of isolated mammalian heart. Although myocardial ischemia and preconditioning is intensely studied in numerous laboratories, there is a lack of information about behaviour of the heart stained with voltage-sensitive dye undergoing ischemic insult.

Thus, the aim of this study was to verify typical preconditioning model in guinea pig and rabbit isolated hearts perfused according to Langendorff which were previously loaded with voltage-sensitive dye (VSD) di-4-ANEPPS. This experimental set-up is used for recording of monophasic action potentials (MAPs) by optical method.

2. Methods

In this study, the hearts of three guinea pigs (average body mass 403±41 grams) and seven New Zealand rabbits of both sexes (1.835±0.320 kg) were included. The guinea pigs were deeply anaesthetized by inhalation of ether, the rabbits by intramuscular application of xylazin (2mg/kg) and ketamin (60mg/kg). The animals were artificially ventilated, their chest opened, the heart excised and its aorta cannulated. The heart was then perfused at constant perfusion pressure (85mmHg) with 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, using Langendorff set-up modified in our laboratory [1]. The solution was oxygenated with 95% O_2 and 5% CO_2. The heart was then placed in thermostat-controlled bath (37°C) filled with Krebs-Henseleit solution. Spontaneously beating hearts were allowed to stabilize at 37°C for 20-30minutes. All preparations exhibiting any dysrrhythmias during this control period were discarded.

The hearts were then loaded with voltage-sensitive dye di-4-ANEPPS ((di-4-amino-naphthyl-ethenylpyridinium; Molecular Probes, Eugene, OR, USA; final concentration for loading 2μM) for 20-25 minutes. In the next step, the dye was washed out for approximately 15 minutes and the myocardium was ready for measurement of monophasic action potentials by optical probe [2].

The optical system consists of a flexible bifurcated

fiber cable with seven optical fibers (six illumination fibers positioned in a circle and a detection fiber positioned in the center of the cable). The fiber 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 artifacts without a need of focusing. The motion artifacts are diminished by slight restriction of the preparation by plastic circle placed around the heart.

The "input" end of the cable with six illumination fibers is connected to a light source. The "output" (detection) fiber is connected to a light detector that senses the beam of emitted light. The optical fibers 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).

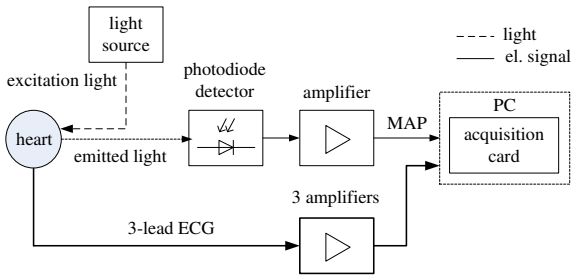

Figure 1. The block diagram of the acquisition system. The excitation light is generated by a light source Intralux DC-1100 with a 150W tungsten-halogen lamp. The light is led by flexible fiber optics to the sample. Fluorescent light is emitted by voltage-sensitive dye present in the sample and led back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

In order to study preconditioning phenomenon, three successive periods of ischemia (global, so-called flow ischemia, e.g. complete stop of perfusion via aortic cannula) and reperfusion were introduced, which lasted 5 minutes (ischemia) and 15 minutes (reperfusion) in the case of guinea pigs, and 10 minutes each in the case of rabbit heart. The protocol is summarized in Figure 2.

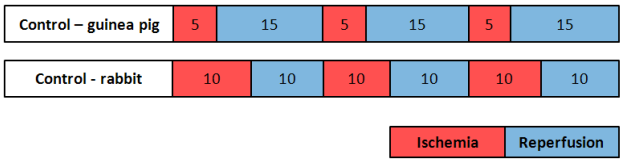

Figure 2. Experimental protocol: top – guinea pig, bottom – rabbit isolated hearts. After 30 minutes of control perfusion, two different preconditioning protocols are applied, with different time of ischemia and reperfusion. For explanation see legend in the right bottom corner.

The recorded electrograms were subsequently analyzed and the severity of arrhythmias was assessed throughout all periods of ischemia and reperfusion according to Lambeth Conventions [4]. Premature ventricular complexes (PVCs) were counted, and the incidence of arrhythmias of various severities was evaluated. Each examined heart was given a score from 0 to 5 (0 – no arrhythmia, 1 – single premature ventricular beats, 2 – salvos, 3 – ventricular tachycardia, 4 – reversible ventricular fibrillation, 5 – sustained

ventricular fibrillation, lasting more than 2 minutes).

The mean coronary flow as a subsidiary parameter was estimated every fifth minute during the whole experiment. It was measured as the outflow of the perfusion solution from the bath in which the heart was placed.

3. Results

In guinea pig hearts, typical changes of the shape of recorded electrogram (mainly ST segment elevation and occurrence of various rhythm disturbances) were observed from the beginning of the first period of ischemia. These changes were quite robust and accompanied by numerous premature ventricular beats (score 2). It was the reason for our decision to introduce ischemia of relatively short duration (5 minutes). Mainly the putative additive effect of di-4-ANEPPS and ischemia, which has not been studied yet, was in play. Another very interesting result is that we did not observe clear attenuation of these electrogram changes during the second and the third ischemic period as it might be expected.

On the other hand, in rabbit hearts loaded with VSD di-4-ANEPPS, the characteristic ischemic changes of electrogram were present at much lower extend and they were accompanied by only a few arrhythmias of lowest severity (score 1). All these symptoms were diminished during consecutive ischemic periods. Rabbit hearts loaded with VSD tolerated ischemia of longer duration (10 minutes) without severe alterations of electrogram. An example of original recording from guinea pig (top) and rabbit (bottom) myocardium during ischemic period is given in Figure 3.

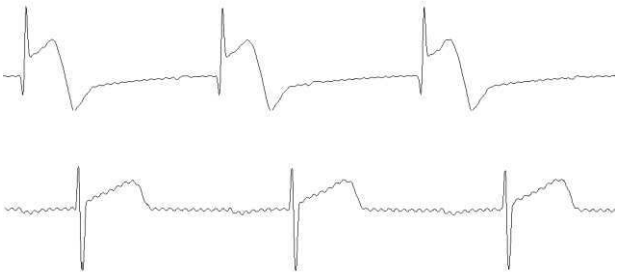

Figure 3. Comparison of (one lead) electrogram recording in guinea pig (top) and rabbit (bottom) isolated heart. The recording was obtained at a comparable moment of ischemia. Note the difference in ST-segment elevation between the preparations.

The most frequent type of arrhythmia in guinea pig hearts during ischemic periods were premature ventricular complexes. For illustration, see Figure 4.

Figure 4. Premature ventricular complex occurring during the onset of ischemic period in isolated guinea pig heart.

Rabbit myocardium responded to ischemic insult with less severe arrhythmias. The most common type of rhythm disturbance which was found during ischemic periods in this preconditioning model is atrio-ventricular (AV) blockade. For example of such recording, see Figure 5.

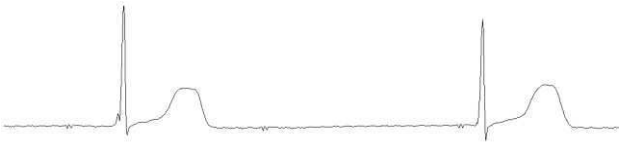

Figure 5. AV-block in ischemic rabbit isolated heart.

When the occurrence of arrhythmias was assessed according to Lambeth score, an apparent difference between the animal models mainly in severity and quantity of rhythm disturbances was visible. Examined guinea pig hearts reached the score 2, rabbit – score 1. When the number of premature ventricular complexes – the most frequent type of arrhythmia during ischemic periods - was compared in consecutive parts of this preconditioning model in rabbits, the attenuation of this symptom of ischemia was clearly visible. PVCs were present at decreasing number in ongoing ischemic periods. In guinea pig, this attenuation was not detectable.

Coronary flow was measured during the whole experiment. However, it served only as a subsidiary parameter, since the coronary flow itself depends on numerous factors and thus it is not possible in such experimental set-up to make any conclusions from this kind of data.

The coronary flow followed the expected pattern in both guinea pig, as well as rabbit hearts – after flow ischemia, at the beginning of reperfusion period(s), increase in mean coronary flow was observed. It is caused by so-called post-ischemic hyperemia. No dramatic decrease in mean coronary flow was found in any period of reperfusion in any of the experiments in either species.

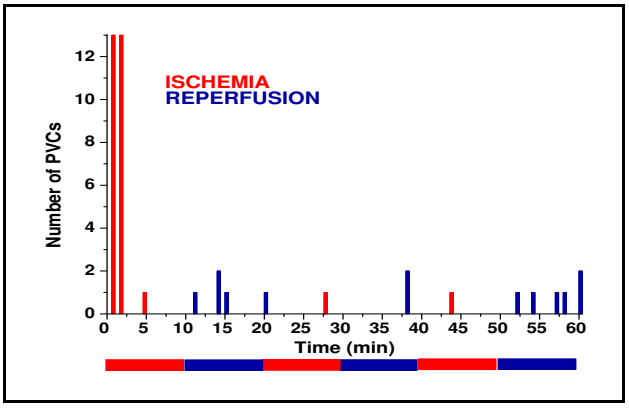

Figure 6. Decreased number of premature ventricular beats during experiment in isolated rabbit hearts. In further ischemic periods, the total number of premature beats is diminished. Only a few arrhythmias appear during reperfusion periods at the end of experiment.

4. Discussion and conclusions

Myocardial ischemia is a prominent diagnosis among the group of cardiovascular diseases. It is accompanied by profound changes in electrical and mechanical properties of the heart muscle which are in clinical practice often observed as changes of electrocardiogram. Clinical as well as experimental studies of this phenomenon and related events are therefore of great importance. One of the related phenomena is preconditioning of cardiac muscle. It is triggered by repeated exposure of the heart to ischemia of various duration and severity. It is believed that preconditioned myocardium is more resistant to further exposure to ischemia [5].

Voltage-sensitive dyes are used for recording of monophasic action potentials (MAPs) by optical probe in a wide range of heart preparations. Optical method of MAPs recording represents advanced approach to the measurement of fine voltage changes on the membrane of cardiac cell. Since late sixtieths of the 20th century when this method was first introduced, it has been noticeably improved and various new voltage-sensitive dyes from numerous chemical groups have been tested. However, many of them were dropped because of their marked side-effects in measured tissue. The exact mechanisms of these side effects remain unknown, although for instance formation of free radicals or direct interaction with the voltage-gated calcium and/or potassium channels has been discussed. The latter may results in altered conductivity and the time-dependent gating of specific ionic channels and thus in diverse electrophysiological disturbances of the cardiac preparation.

In our previous experiments, we have found profound effects of VSD di-4-ANEPPS on guinea pig and rabbit heart muscle [6]. Mainly the electrical disturbances during the loading with VSD were observed – mainly numerous arrhythmias and spontaneous heart rate slowing. These effects were present in both species tested, however in rabbit hearts to much lower extend.

The information about additive effect of myocardial ischemia and VSD loading are missing in the literature. Based on our present experiments, we can conclude that although the procedure of dye loading affects electrophysiological properties of the myocardium in both species, in case of rabbit heart it is negligible (it is quite resistant to side-effects of VSDs). In guinea pig mycardium, more cautiousness is needed. Thus, rabbit myocardium is more suitable for studying myocardial ischemia in the model where the heart muscle is loaded with VSD.

Acknowledgements

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