Voltage Sensitive Dye di-4-ANNEPS Prolongs Impulse Conduction through Ventricles, but not through AV Node in Isolated Rabbit Heart

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Abstract

Voltage sensitive dyes are widely used for recording of action potential in various cardiac studies. The elementary condition for its application is the fact that measured electrophysiological parameters would not be affected by the measuring procedure itself. The RR interval prolongation in the presence of the most frequently used voltage sensitive dye di-4-ANEPPS has been reported, but its exact mechanism is not known.

In this study, the impact of di-4-ANEPPS on impulse conduction through the atrioventricular (AV) node and through cardiac ventricles in isolated rabbit heart was examined. The impulse conduction through AV node in the presence of di-4-ANEPPS was prolonged only during dye loading in the 14th, 16th and 17th minute. Only sporadic AV blocks were observed. In comparison, the prolongation of impulse conduction through cardiac ventricles was observed from the 13th minute of loading and persisted to the end of this phase as well as over the whole wash out phase.

1. Introduction

Voltage sensitive dye (VSD) represents an interesting tool for the study of cardiac electrical activity. It allows monitoring of action potential from selected area of the heart surface [1]. The recording is based on excitation of the dye molecules bound to the cell membrane under fluorescent light. Exposing of the tissue to the excitation light with preselected spectrum results into the excitation of the molecules to higher energetic state and subsequent light emission. The intensity of fluorescence light emitted by VSD is proportional to the transmembrane potential [2].

The elementary condition for VSD application is the fact that measured electrophysiological parameters would not be affected by the procedure itself. However, the evidence about influence of cardiac electrophysiological parameters by the most frequently used VSD, di-4-ANEPPS, does not exist. Our laboratory previously showed the decrease of heart rate in rat, guinea pig and rabbit isolated hearts in the presence of di-4-ANEPPS [3]. Nevertheless, the exact mechanisms of influencing cardiac impulse generation or its spreading through the heart tissue in the presence of di-4-ANEPPS are still not known. The slowing of cardiac impulse propagation in isolated guinea pig heart under di-4-ANEPPS was described by Larsen et al. [4]. PQ interval prolongation and transient block of atrioventricular conduction in rat heart were reported by Nygren et al. [5].

In this study, the effects of di-4- ANNEPS on impulse conduction through AV node and through the ventricles in isolated rabbit hearts were examined.

2. Methods

2.1. Isolated heart preparation

All experiments were carried out according to the guidelines for animal treatment approved by local authorities and conformed to the EU law. Twelve adult New Zealand rabbits (both sexes) were included in this study. Animals were anesthetized by intramuscular application of xylazin (2 mg/kg) and ketamine (60 mg/kg) and artificially ventilated. The bilateral thoracothomy was performed and the heart was excised and placed into a bath containing cold Krebs–Henseleit solution (4 °C). The aorta was cannulated and the heart was fixed to a modified Langendorff set-up [6] and perfused at constant pressure (80 mmHg) with Krebs-Henseleit solution (NaCl, 118 mM; $NaHCO_3$, 24 mM; KCl, 4.2 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.25 mM; glucose, 5.5 mM). The perfusing solution was aerated with 95% O_2 and 5% and the temperature was maintained constant at 37 °C for all experiment.

2.2. Data acquisition

Spontaneously beating hearts were allowed to stabilize

for 20 -25 minutes. Three orthogonal electrograms were recorded using touch-less bipolar electrode system (three pairs of Ag-AgCl disc electrodes) placed in the wall of the bath [7].

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 2000 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.

After stabilization period, 6 hearts were subjected to experimental protocol consisting of 20 minutes of staining with di-4-ANEPPS (di-4-amino-naphthyl-ethenyl-pyridinium; Molecular Probes, Eugene, OR, USA; final concentration for loading 2 μM), followed by 20-25 minutes of wash out of redundant dye by Krebs-Henseleit solution. Another 6 hearts subjected to 40-45 minutes of perfusion without di-4-ANEPPS immediately after stabilization were used as control.

The recorded electrograms were subsequently analysed: PQ interval changes and incidence of AV block of the second and the third degree were followed for assessment of impulse conduction through AV node. For assessment of impulse conduction through the ventricle, the QRS complex duration was analysed.

3. Results

Comparison of the PQ and QRS duration from electrograms recorded during stabilization, loading and wash out in experiments with and without VSD application was performed using non-parametric Mann-Whitney test (with the significance level $\alpha = 0.05$). Mean and STD values of PQ and QRS duration at the end of each period and the result of comparison are shown in Table 1.

Table 1. Values (mean ± STD) of PQ and QRS duration (in ms) and results of comparison Mann-Whitney test between two groups of data. *- significant difference.

PQ duration			
	Without VSD	With VSD	p value
Stabilization	52.6±6.5	55.4±7.4	0.4674
Loading	52.3±7.8	57.1±6.9	0.2471
Wash out	54.4±7.9	58.5±8.3	0.4231
QRS duration			
	Without VSD	With VSD	p value
Stabilization	21.9±1.1	25.1±3.9	0.1282
Loading	22.1±1.9	27.7±5.2	0.0309*
Wash out	23.6±2.1	28.6±4.6	0.0093*

The box plots of PQ and QRS duration values for each minute of stabilization, loading and wash out periods of the experiments with and without di-4-ANEPPS application are shown in Fig.1-3. VSD significantly prolongs PQ duration in the 14th, 16th and 17th minute of dye loading phase and QRS duration from the 13th minute of dye loading phase as compared with control hearts (see Fig. 2).

During wash out period, changes in QRS duration persist, while changes in PQ period were reverted (see Fig. 3). AV blocks of the second and the third degree were detected only sporadically.

Observed changes in QRS duration during the first three minutes of experiment were attributed to the stabilization of the hearts.

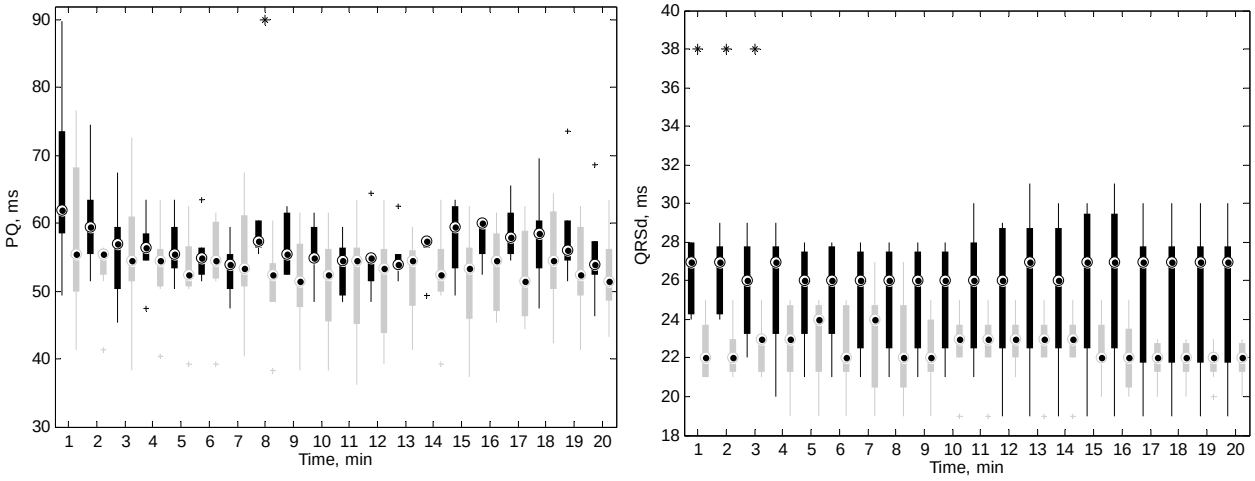

Figure 1. Box plots of PQ (left part) and QRS (right part) duration during stabilization period. Data from experiments with and without di-4-ANEPPS are depicted with black and gray, respectively. * for $p<0.05$ (Mann-Whitney test).

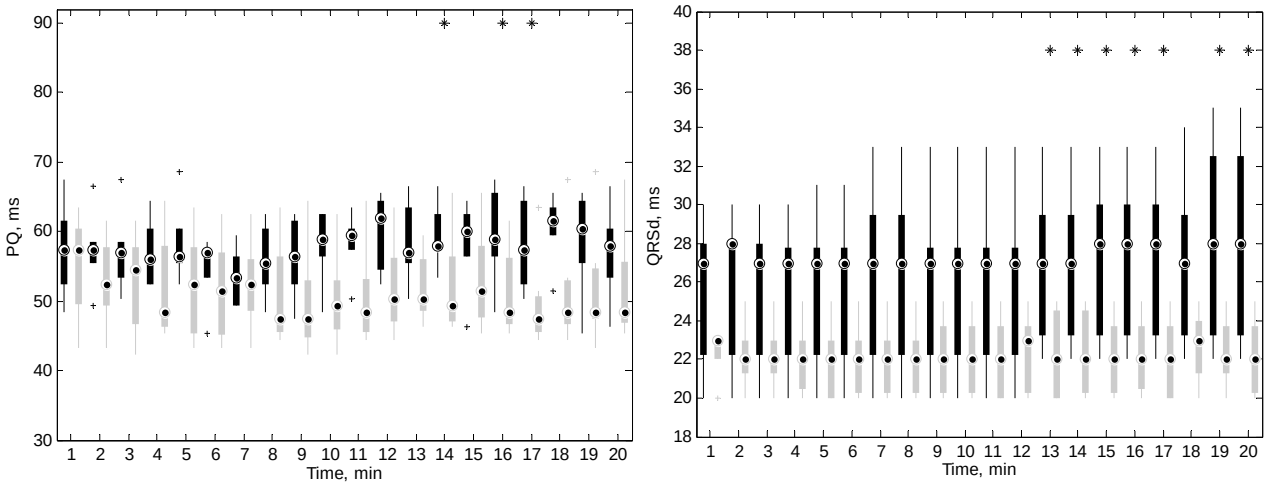

Figure 2. Box plots of PQ (left part) and QRS (right part) duration during dye loading. Data from experiments with and without di-4-ANEPPS are depicted with black and gray, respectively. * for $p<0.05$ (Mann-Whitney test).

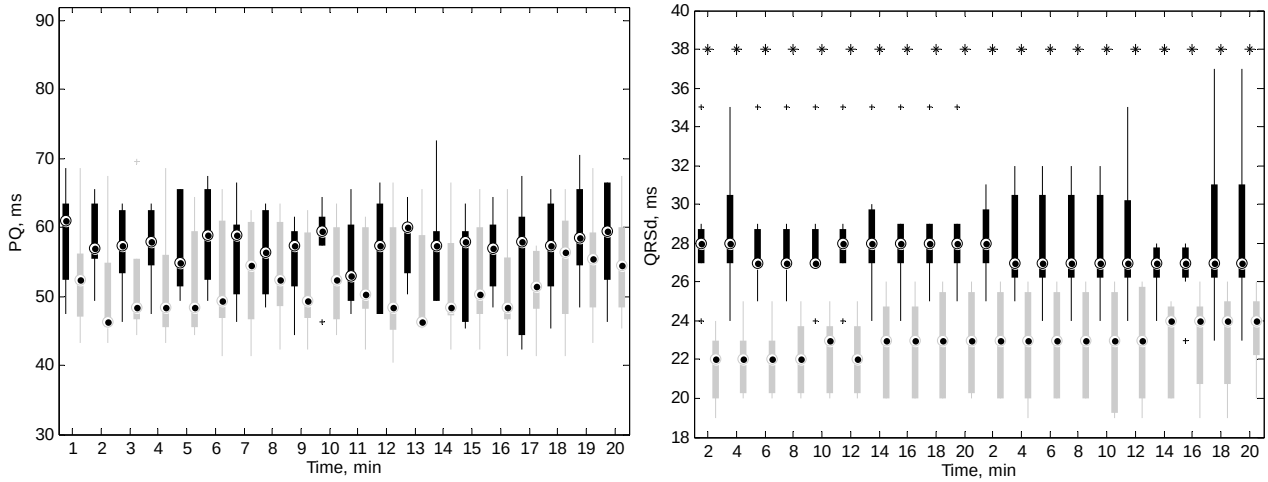

Figure 3. Box plots of PQ (left part) and QRS (right part) duration during wash out. Data from experiments with and without di-4-ANEPPS are depicted with black and gray, respectively. * for $p<0.05$ (Mann-Whitney test).

4. Discussion

Voltage sensitive dye can be used for action potential monitoring from selected area of the heart surface. Since the introduction of this method, many VSDs were presented and tested. Although the impact of di-4-ANEPPS on electrophysiological parameters is disputable, this dye is still the mostly used VSD for cardiac studies. The slowing of heart rate in the presence of di-4-ANEPPS in rat, guinea pig and rabbit hearts was previously described in our laboratory [3]. There is an evidence for influence of di-4-ANEPPS on cardiac impulse conduction through heart ventricles as well as AV node in various animal models. Slowing of the conduction velocity in longitudinal as well as transversal direction was described in isolated guinea pig heart [4]. In rat heart, influence of the di-4-ANEPPS on conduction through AV node as well as conduction through the ventricles was observed. The PQ interval prolongation and even transient block of AV conduction [5] as well as QRS complex prolongation were described [8].

In this paper, we show that in isolated rabbit heart di-4-ANEPPS prolongs PQ duration only in negligible time period during the dye loading phase. The prolongation of the QRS complex duration is present from the 13th minute of dye loading till the end of this period and subsequent

wash out. These results suggest that in isolated rabbit heart (besides the rat heart, where di-4-ANEPPS affects both conduction of cardiac impulse in ventricles as well as AV node), di-4-ANEPPS prolongs impulse conduction through ventricles, but only very slightly affects impulse conduction through AV node. These results are in good agreement with previously described different sensitivity to di-4-ANEPPS among various animal species [9].

5. Conclusion

It can be concluded, that in isolated rabbit heart, voltage sensitive dye di-4-ANEPPS leads to prolongation of cardiac impulse conduction through the ventricles, although conduction through AV node is impaired only insignificantly.

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