MODELING AND ESTIMATION OF ELECTRICAL PARAMETERS OF EQUIVALENT CIRCUIT IN ISOLATED RAT HEART

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Abstract: Analysis of complex impedance plots enables to fit electrical models to biological phenomena. At least three factors effect on estimation of parameters of equivalent circuit: choice of type of electrical model, choice of fitting method and choice of statistical analyse method. In this study the model of parallel extracellular resistance with serial intracellular resistance and membrane capacitance has been fitted to experimental data obtained from n=20 isolated rat hearts during normoxia in the range of frequency from 1.25 kHz to 206 kHz and electrical parameters of equivalent circuit were estimated. Fitting process based on Simplex method. Modeling procedures and statistical analyses were done due to STATISTICA software. The following values of extracellular resistance R_{ext}, intracellular resistance R_{int} and membrane capacitance C_m were found after fitting equivalent circuit to subgroup of n=12 subjects: R_{ext}=41.96 ohm (CI: 34.41÷51.03 ohm), R_{int}=166.72 ohm (CI: 97.62÷314.62 ohm) and C_m=71.79 nF (CI: 24.26÷241.94 nF).

Introduction

Application of impedance spectrometry for measurement of electrical properties of biological tissues is well known and some examples of applications are widely described in [1]. Analysis of complex impedance plots enables to fit electrical models to biological phenomena. At least three factors effect on estimation of parameters of equivalent circuit: choice of type of model, choice of fitting method and choice of statistical analyse method, from which two last are discussed. In this study the model of parallel extracellular resistance with serial intracellular resistance and membrane capacitance that is presented in Figure 1, has been fitted to experimental data obtained from isolated rat hearts in the range of frequency from 1.25 kHz to 206 kHz and electrical parameters of equivalent circuit were estimated. This simplified circuit represents the base structure of cells and tissues and allows quite good to describe electrical behavior of biological tissue. It is generally used for modeling parts of human body by equivalent circuit [2-4] and in this pilot study it was applied to estimate values of parameters of equivalent circuit for isolated rat heart during normoxia.

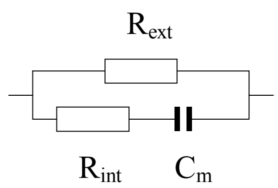

Figure 1: Equivalent circuit of a cell or a tissue: R_{ext} – extracellular resistance, R_{int} – intracellular resistance, C_m – membrane capacitance

Materials and Methods

The experiments were performed on 20 male Whistar rats (200 – 300 g) fed ad libitum. They were injected with 1000 i.u. of heparin and anesthetized with sodium pentobarbital in dose 10 mg/kg. After thoracotomy the heart was removed and placed in an ice-cold Krebs-Henseleit buffer, and the cannula was inserted into aorta. The isolated heart was perfused by Langendorff technique at a constant pressure of 76 cm H_20 with Krebs-Henseleit bicarbonate buffer containing (in nM): 118.0 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 11.0 glucose. The buffer was equilibrated with 95% O_2 and 5% CO_2 gas mixture giving pH 7.35-7.40 and pO_2 550-580 mm Hg at 37°C.

Measurements of impedance modulus and phase angle were conducted after 15 min. of perfusion by using a modified impedance spectrometer [5]. The impedance spectrometer based on tetrapolar current method and enabled measurement of complex impedance of rat heart in the range of frequency from 1.25 kHz to 206 kHz. Impedance modulus and phase angle were measured at following step values of frequencies: 1.25, 2.0, 3.0, 3.9, 4.7, 5.6, 6.5, 10.5, 14.0, 14.5, 18.5, 30.0, 45.0, 60.0, 74.0, 85.0, 107.0, 160.0, 202.0, 206.0 kHz. Next resistance and reactance were calculated from experimental data.

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Then fitting procedure was applied to find parameters of equivalent electrical circuit. Fitting method based on usage of imaginary part of impedance to find the best fit. Parameters of fitted reactance curve and resistance data were applied to calculate values of extracellular resistance, intracellular resistance and membrane capacitance of examined tissue.

It was Simplex method used as a fitting method. For iterative process assumed least square procedure as a loss function and maximum iteration equal 100. At the beginning initial values were taken from model with known resistances and capacitance to calibrate measurement device and next from Cole-Cole plots for each data sets. This method was applied for data collected from individual subjects and for average data derived respectively from 20, 17 and 12 subjects (exclusion criteria will be described with results of research). All modeling and fitting procedures were done due to STATISTICA software.

Results

As it was said the purpose of the study was to estimate values of parameters of equivalent circuit for isolated rat heart during normoxia. The preliminary analyses were to check possibility of usage Simplex method to fit experimental data to reactance spectrum in the range of frequency 1.25 kHz to 206 kHz (20 measurement points) for single isolated rat hearts and also for average data.

The aim of this stage of research was estimation of the accuracy and validity of iterative fitting process. There were two parameters applied as indicators of goodness of model fit. One of them was linear regression coefficient between observed and predicted values, and the other was determination coefficient.
For group of 20 individual subjects the values of determination coefficient were above 79.4% for 75% of cases, and greater than 89.6% for 50% of them.

The results of fitting reactance curve to experimental data by Simplex method for selected single heart are shown in Figure 2 and Figure 3.

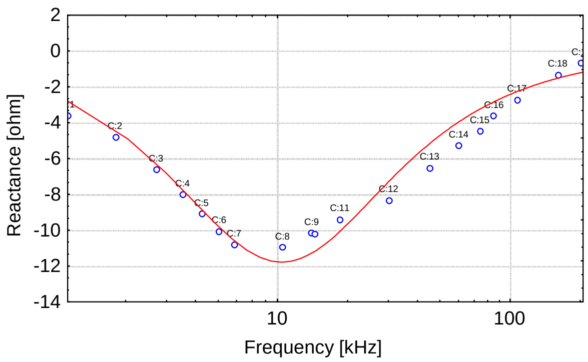

Figure 2: The experimental data (c_1-c_{20}) versus fitted data (red line) for single isolated rat heart, R^2=94.86%

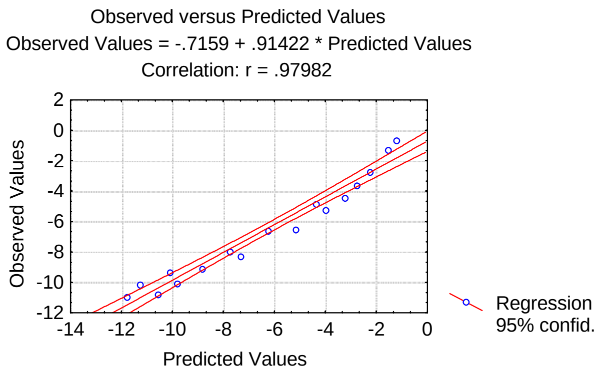

Figure 3: The linear regression curve between observed and predicted reactance data for single isolated rat heart

Figure 2 shows experimental data versus fitted data and Figure 3 presents linear regression between observed and predicted values of reactance for the same heart. The linear regression coefficient in this case is equal 0.97982 and determination coefficient is respectively 94.86%.

For comparison, the results of experiments for average data from 20 isolated rat hearts are shown in Figure 4. The fitted reactance curve lays above experimental data in the range of frequency 1.25 kHz - 6.5 kHz and 30 kHz -206 kHz, and below experimental data in the range of frequency 6.5 kHz - 30 kHz. The linear regression coefficient for fitted curve for average reactance spectrum is equal 0.93285, and determination coefficient is 54.41%.

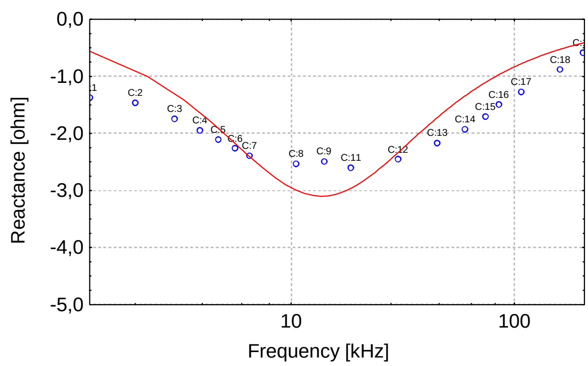

Figure 4: The experimental data (c_1-c_{20}) versus fitted data (red line) for average reactance curve from isolated rat hearts (n=20), R^2=54.41%

Data derived from fitting process enabled to calculate parameters of equivalent circuit. The values of extracellular resistance R_{ext}, intracellular resistance R_{int} and membrane capacitance C_m, calculated from fitted reactance curve and resistance curve are presented in Table 1.

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Table 1: Parameters of equivalent circuit fitted to average experimental data (n=20)

R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
44.15	320.93	33.00

Although the parameters of equivalent circuit were able to calculate, the next question was how well these parameters described the isolated rat heart with its variability between subjects in normoxia. To answer the question the goodness of data fit was revised once again. During preliminary analyses occurred that the value of determination coefficient for average data (n=20) was only about 54.41% as if for 75% of single hearts it was above 79.42%.

One of the reasons of this phenomenon was fact that data exhibited a wide dispersion and they did not have gaussian distribution (Shapiro-Wilk test was used to check normality of distribution). The other reason was influence of impedance spectrometer accuracy on goodness of fit. It were observed very small values of phase angle (approximately 0.1°) during measurements of impedance modulus and phase angle for frequency above 107.0 kHz in several cases. Exclusion those measurement points from fitting process improved fit and enabled to achieve better determination coefficient for those hearts from one hand, but from the other hand it caused changes in analyse protocol. To solve the problem of maintaince the same analyse protocol (20 measurement points for each fitted data set) an exclusion criterion was applied that rejected 3 cases with determination coefficient below 50% from further analyses.

According to these assumptions the next step was analysis and estimation parameters of equivalent circuit for n=17 subjects and usage medians instead of means in further analyses. The values of extracellular resistance R_{ext}, intracellular resistance R_{int} and membrane capacitance C_m, calculated from fitted reactance curves and resistance curves for 17 isolated rat hearts are presented in Table 2.

Table 2: Parameters of equivalent circuit (medians) fitted to experimental data (n=17)

	R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
Median	46.21	292.88	61.17
Confidence Interval	31.13÷52.41	131.51÷418.82	13.15÷159.12

The next analyses included 12 cases from those 17, for which intracellular resistance R_{int} to extracellular resistance R_{ext} was below 10. It was done to improve the reliability of findings. It seemed during experiments that all subjects, who were characterized by this coefficient's value, belonged to the same subgroup. This exclusion criterion was assumed to verify values of parameters of equivalent circuit for isolated rat heart examined in normoxia. In further study it can change, because this pilot describing study did not allow estimating the state of heart after examination.

The results of data analyse and evaluating parameters of equivalent circuit for n=12 isolated hearts are shown in Figure 5 and in Table 3. Figure 5 presents box and whisker plots for external resistance R_{ext}, internal resistance R_{int} and membrane capacitance C_m for isolated rat heart.

The least variability range among all calculated parameters is observed for extracellular resistance R_{ext}, which is the one of parameters of electrical equivalent circuit, which is easy to estimate.

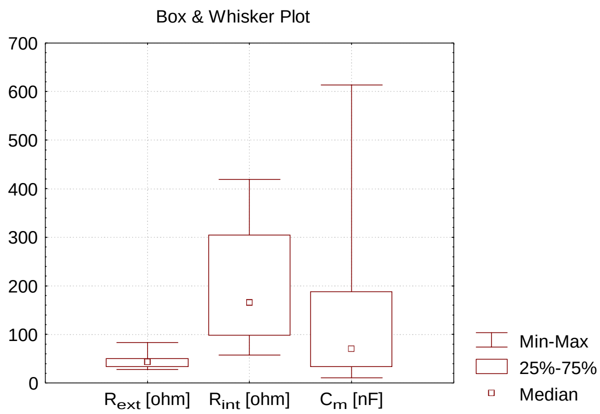

Figure 5: The box and whisker plots for external resistance R_{ext}, internal resistance R_{int} and membrane capacitance C_m for isolated rat heart (n=12)

Table 3: Parameters of equivalent circuit (medians) fitted to experimental data (n=12)

	R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
Median	42.91	165.12	70.57
Confidence Interval	31.13÷51.03	97.62÷314.62	24.26÷241.94

The variability range is the smaller the more homogeneous is group of analysed data as it is shown in Figure 6 that presents comparison between estimated parameters of equivalent circuit respectively for 17 and for 12 cases.

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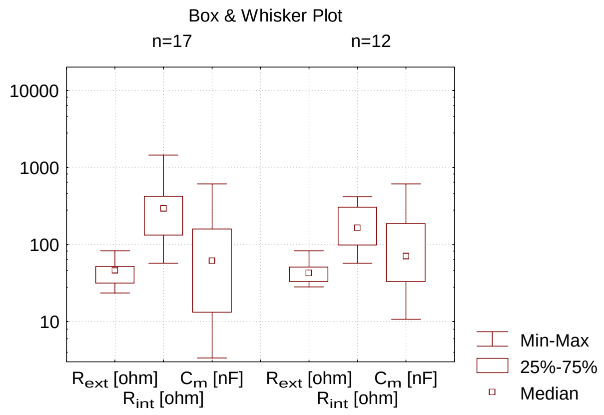

Figure 6: Comparison between external resistance R_{ext}, internal resistance R_{int} and membrane capacitance C_m for 2 groups of isolated rat hearts: (n=17) and (n=12)

As it was examined during primary inspection, data sets did not have normal distribution – data derived from estimation process had a positive skewed distribution. So according to [6] additional method of analyses was applied to describe and compare results of estimating equivalent circuit parameters. Raw data were transformed to achieve data, which have quasi-gaussian distribution. In this particularly case logarithmic transformation was applied. The data after taking logarithms for n=12 subjects are presented in Figure 7. As one can see the transformed data have a more nearly symmetric distribution.

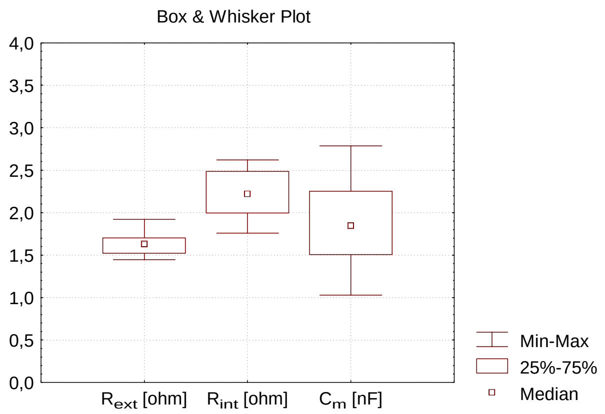

Figure 7: The box and whisker plots for external resistance R_{ext}, internal resistance R_{int} and membrane capacitance C_m for isolated rat heart (n=12). Values of R_{ext}, R_{int} and C_m in logarithmic scale.

After transformation data were analysed once again and a quantity known as the geometric mean was used to describe results of estimation. The results of evaluating parameters of equivalent circuit for 17 isolated rat hearts are shown in Table 4 and for 12 subjects respectively in Table 5.

Table 4: Parameters of equivalent circuit (geometric means) fitted to experimental data (n=17) after transformation process

	R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
Geometric mean	42.54	262.22	48.83
Confidence Interval	34.95÷51.78	158.94÷432.59	22.08÷108.00

Table 5: Parameters of equivalent circuit (geometric means) fitted to experimental data (n=12) after transformation process

	R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
Geometric mean	41.96	166.72	71.79
Confidence Interval	34.41÷51.03	97.62÷314.62	24.26÷241.94

Table 6 shows that in this case log transformation and obviously "back-transformation" successfully removed skewness – the geometric mean is similar to the median and it is also less than mean of the raw data.

Table 6: Parameters of equivalent circuit (medians & means) fitted to experimental data before (-) and after (+) transformation process (n=17 & n=12)

a. medians

Number of observation	Transformation process	R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
17	-	46.21	292.88	61.17
17	+	46.21	292.88	61.17
12	-	42.92	165.12	70.57
12	+	42.79	165.00	69.94

b. means for raw data and geometric means for transformed and back-transformed data

Number of observation	Transformation process	R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
17	-	45.56	416.08	116.48
17	+	42.54	262.22	48.83
12	-	43.98	201.23	137.03
12	+	41.96	166.72	71.79

The purpose of the last group of analyses was to compare parameters of equivalent circuit after transformation process to parameters calculated for averaged curve respectively for n=17 and n=12 subjects. The results of this stage of study are shown in Table 7.

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Table 7: Parameters of equivalent circuit fitted to experimental data after transformation process (+) (geometric mean values) and fitted to averaged curve (avc) (n=17 & n=12)

Number of observation	Transformation process	R_{ext} [ohm]	R_{int} [ohm]	C_m [nF]
17	+	42.54	262.22	48.83
17	avc	43.78	241.87	41.86
12	+	41.96	166.72	71.79
12	avc	41.87	162.39	65.74

Concluding, the more homogeneous is group of analysed data the difference between values of geometric mean and values of parameters calculated for averaged data is the smaller.

Discussion

During preliminary data analyses it occurred that Simplex method used as a fitting method in iterative process provided high fitting of collected data to examined model (determination coefficient above 79.4% for 75% of subjects), although in this research it based only on usage of imaginary part of impedance from 20 measurement points.

In this study parameters of equivalent circuit were estimated from data derived for single isolated rat heart also for averaged data (often used) to compare and verify the values of parameters. It occurred that statistical methods based on assumption about normality of data distribution cannot be used to analyse collected data. Asymmetric distribution of data with positive skewness does not allow using arithmetic mean as a parameter, which describe collected data.

Statistical analyses conducted on data sets derived from measurements of impedance modulus and phase angle show that only median and geometric mean are good indicators of variability of parameters of equivalent circuit and both can be use to estimate their values. For subgroup of 12 subjects the values of median and geometric mean for extracellular resistance R_{ext}, intracellular resistance R_{int} and membrane capacitance C_m are similar.

Confidence intervals calculated for extracellular resistance R_{ext}, intracellular resistance R_{int} and membrane capacitance C_m show the wide variability range for all estimated parameters. It indicates that examined group of subjects is distinguished by high variability within subjects that could be caused by such factors as heterogeneity, anizotropy and differences in dimensions of anatomical structures of measured tissue for individuals.

Conclusions

The results of analyses confirmed that equivalent circuit of parallel extracellular resistance with serial intracellular resistance and membrane capacitance could be used to model electrical behavior of isolated rat heart in normoxia.

The complex impedance plots obtained for isolated rat hearts make possible to model and to fit equivalent circuit to experimental data by using standard statistical software.

Only high homogeneous group of subjects can provide good estimation of parameters of equivalent circuit.

Electrical parameters evaluated from fitted curves for normal myocardium tissue can be useful in further examination of electrical properties of biological tissues, especially in comparison to electrical properties of pathological tissue e.g. ischemic tissue.

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