SUPPLEMENTAL MATERIAL

Measurements of longitudinal impedance and calculation of gap junction resistance

The longitudinal impedance of atrial preparations (≤1 mm diam. 5-6 mm length) was measured by constraining alternating current to flow along the intracellular pathway.\(^1\) A three-chambered bath (4.5 cm x 2 cm x 1 cm) (Supplemental Figure 1), separated by rubber membranes, was used, and the preparation was pulled through tight holes in the membranes with at least 1 mm protruding into the outer chambers. The length of muscle in the central chamber was 2.5 mm. The central chamber contained mineral oil, and the outer chambers contained Tyrode's solution at 37ºC. Alternating current (0.25-150 kHz) was passed between platinum (Pt)-black electrodes in the outer chambers to constrain current to the intracellular pathway of the muscle within the oil-gap, with a fraction through a parallel extracellular shunt. The frequency-dependent intracellular resistance (\(r\)) of the preparation was recorded with a balanced Wien bridge (Wayne-Kerr, Wetherby, UK). The bridge maintained a constant 10 mV peak-to-peak signal across the preparation, permitting the system to be analysed as a lumped circuit. We avoided a non-voltage-clamped arrangement as has been used by some others when analysing the impedance properties of skeletal muscle.\(^2,3\)

It was important to avoid an hypoxic core to the preparation because this would falsely increase \(R_i\). Several lines of evidence indicated that this was so: (1) the preparation diameter was <1 mm; (2) impedance measurements were stable for at least 30 minutes and (3) tissue structure, examined by histology and immunohistochemistry, and metabolic integrity, examined by measuring intracellular [ATP], are unaffected by experiments.\(^4\)
Prior to mounting specimens in the Perspex bath the Pt-black electrode resistance, \( r_p \), and capacitance, \( c_p \), were measured separately in a large volume of Tyrode’s solution. Experimental measurements were performed using alternating current (20 Hz - 300 kHz). Two complete sets of experimental recordings of preparation impedance and phase angle were performed at 10-minute intervals; values always agreed within 10\%, and average values were used.

\( r_p \) and \( c_p \) are considered to lie in series but the bridge reads the values as if they are in parallel. In order to obtain their values the following relationships were used.

\[
r_p = \frac{1}{g(1+Q^2)} \quad \text{[7]}
\]

\[
c_p = c (1+1/Q^2) \quad \text{[8]}
\]

Where \( r_p \) and \( c_p \) are the polarization resistance and capacitance of the electrodes and \( g \) and \( c \) are the values obtained from the Wein bridge: \( G \) (conductance) is the inverse of \( R \) (resistance). The constant \( Q \) has a value of \( \omega c/g \), where \( \omega \) is the radial frequency of the measurement (i.e. \( \omega = 2\pi f \)).

For impedance measurements of the specimen, the electrode resistance and capacitance were subtracted from the total measured values by use of the network shown in supplemental figure 2. where:

\[
r = \left[ 1 + (r\omega c) \right] \left[ r_p + r_s/ \left[ 1+ (r\omega c)^2 \right] \right] \quad \text{[9]}
\]

and,

\[
1/\omega c = \left[ 1 + 1/(r\omega c) \right] \left[ 1/ \omega c_p + (1/\omega c_s)/ \left[ 1+(1/r\omega c)^2 \right] \right] \quad \text{[10]}
\]
the subscript $p$ refers to polarization (electrode) values and $s$ refers to sample values. The unsubscripted values are taken directly from the bridge.

If $\omega c < 1$ and $r_p < r_s$, which was always the case, then equations [9] and [10] can be approximated by

$$ r = r_s + r_p \quad \text{or} \quad r_s = r - r_p \quad [11] $$

$$ c_s = c - 1/ \omega^2 r^2 c_p \quad [12] $$

Lastly the interelectrode capacitance, $c_x$, measured between the outer chambers with the preparation removed from the middle chamber, was subtracted from $c_s$ as the two were assumed to lie in parallel.

The impedance of the system, $z$, can be expressed by;

$$ z = (r + jx) \quad [13] $$

Where $r$ is the resistance and $x$ the reactance; $j$ is the complex operator $\sqrt{-1}$. The reactance of a capacitor is $-1/\omega c$ so that the admittance, $y$, ($=1/z$) can be expressed as;

$$ y = (g + j\omega c) \quad [14] $$

Thus, to obtain the impedance values of myocardium, the measured values of $c_s$ and $g_s$ were converted to $r$ and $-x$ values by the following relationships;

$$ r = g/(g^2 + (\omega c)^2) \quad [15] $$

$$ -x = \omega c/ (g^2 + (\omega c)^2) \quad [16] $$

These values could then be expressed in the form of an $r$ versus $-x$ plot which provided a locus for every separate time constant.
The results were then manipulated to fit a network where the impedance, \( z \), was modelled as 
\[ z = (z_i, r_{ec})/(z_i+r_{ec}), \]
where \( r_{ec} \) is the resistance of the extracellular shunt and \( z_i \) is the impedance of the intracellular pathway (Supplemental Figure 3). \( r_{ec} \) was measured separately by measuring the resistance between two Pt-black needle electrodes a known distance apart in the muscle within the oil-gap.

Longitudinal impedance, \( z_i \), was analyzed as two series components; cytoplasmic resistivity, \( r_c \), and junction impedance, \( z_j/(z_j+r_c) = z_i \). \( z_i \) values were expressed as their resistance, \( r_s \), and reactance, \( -x_s \), components, i.e. \( z_i = r_s + jx_s \).

When measurements were completed the length and radius of the preparation in the oil-gap were measured. Lower case values of variables (\( r, x \), expressed as \( \Omega \cdot \text{cm}^{-1} \)) were converted to specific (\( R, X, \Omega \cdot \text{cm} \)) values by scaling to the cross-sectional area (CSA) of each preparation and the proportion of CSA occupied by muscle. The non-muscle fraction of CSA was calculated from the value of \( r_{ec} \), assuming it was filled with Tyrode’s solution (resistivity, 49 \( \Omega \cdot \text{cm} \)). Repeated measures from adjacent preparations from the same heart showed less than 10% variability regardless of the chamber studied.

**Analysis and interpretation of data**

Supplemental Figure 4 shows a plot of resistance, \( R_i \), as a function of frequency between 20 Hz and 300 kHz. \( R_i \), declines with increasing frequency, levelling off toward a constant finite value at higher frequencies. The plot is interpreted as one or more parallel \( r_c \) circuits in series with a resistance. For each \( r_c \) circuit with a different time constant (\( \tau = r_c \)), there will be a specific range of frequencies over which the impedance will decline.

More accurate analysis was obtained by plotting, at each frequency, the resistive (\( R_s \)) and reactive (\( -X_s \)) components of \( Z_s \) as a function of each other. Supplemental Figure 5 shows
such plots using the data of Supplemental Figure 4; points for lowest frequencies are on the right side, and each time constant shows as a separate semicircular locus. Semicircles were fitted to the left (higher frequencies) loci, and the intercepts with the $R_s$ axis are shown as $R_1$ and $R_2$. The plots were analyzed in terms of circuit elements in the longitudinal pathway of the muscle preparations, in parallel with a resistive shunt, $R_{ec}$, which is present in the extracellular space of the preparation and in the thin layer of Tyrode's solution adhering to the muscle beneath the oil in the central chamber.

Such plots do not specifically display frequency information, but the values of some frequencies are shown on the plots. The time constant, $\tau$, of the parallel $\text{rc}$ circuit generating a particular dispersion is obtained from the relationship $2\pi f^* \tau = 1$, where $f^*$ is the frequency generating the maximum value of $-X_s$ in the locus. The low-frequency dispersion exhibited a maximum reactance at $\approx 40$ Hz, equivalent to a time constant of $\approx 4$ ms and similar to that of the myocardial membrane time constant. Thus, the low-frequency dispersion was interpreted as resulting from the surface membrane of the preparation in the outer chambers, $R_m$ and $C_m$ in Supplemental Figure 3.

The high-frequency dispersions (maximum reactance at 10 to 40 kHz) have been interpreted as a junction impedance between cells in the longitudinal pathway. Agents such as heptanol leave the $R_1$ unchanged whilst increasing the $R_2$ value in Supplemental Figure 5. The residual resistance at the higher frequencies was considered to result from the resistance of the sarcoplasm.

Supplemental Figure 3 shows an equivalent circuit that was used to analyze the $-X_s/R_s$ plots of Supplemental Figure 5. Included in the circuit is a shunt resistance, $R_{ec}$, representing current flow through the extracellular compartment of the preparation in the oil gap. The low-frequency intercept of the left dispersion with the resistance axis, $R_2$, is a parallel combination...
of $R_{ec}$ and the total intracellular resistivity, $R_i$; where $R_i$ is the sum of $R_c$ and $R_j$. The high-
frequency intercept, $R_1$, is a parallel combination of $R_{ec}$ and $R_c$ alone. The difference, $R_2-R_1$, will therefore be a function of $R_j$ and $R_{ec}$. The values of the intercepts $R_1$ and $R_2$ were determined in all preparations, along with the preparation length in the oil gap, total CSA (including the adherent layer of Tyrode's solution), and the proportion of CSA occupied by muscle for calculation of the specific resistances in units of $\Omega$.cm. There were no significant differences in preparation dimensions between each of the four experimental groups.

References


**Figure Legends**

Supplemental Figure 1. Diagram of the three-chambered impedance bath (not to scale). The outer chambers contained oxygenated Tyrode’s solution (37°C) and the middle chamber contained mineral oil gel. Preparations were pulled through holes in the rubber membranes. Alternating current was passed across preparations via Pt-black electrodes, one in each outer chamber.

Supplemental Figure 2. Model of network used for impedance measurement. $c =$ capacitance, $r =$ resistance, $c_p =$ polarisation capacitance, $r_p =$ polarisation resistance, $r_s =$ sample resistance, $c_s =$ sample capacitance.

Supplemental Figure 3. Equivalent circuit of the muscle preparation used to analyze experimental data. $r_m =$ membrane resistance; $c_j =$ junctional capacitance; and $c_m =$ membrane capacitance.

Supplemental Figure 4. A plot of intracellular resistance, $R_i$, as a function of measuring frequency. The dotted lines represent the frequency-independent values of $R_i$ at high and low frequencies. The smaller value at high frequency corresponds to cytoplasmic resistivity, $R_c$; the difference between the two values is a function of gap junction resistance, $R_j$.

Supplemental Figure 5. Analysis of intracellular impedance in terms of resistive, $R_s$ and reactive, $X_s$. The left semicircular dispersion was fitted to a plot of $R_s$ against $-X_s$ at frequencies between 0.02 to 100 kHz using the equation $X_s = a^2 - (R_c + b) - c$, where $a$, $b$, and $c$ are constants. Intercepts on the abscissa are measures of $R_c$ ($R_1$) and $R_i$ ($R_2$).
Supplemental Figure 1

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Supplemental Figure 2

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