FIXED CHARGES OF THE HEART MUSCLE INTERSTITIUM

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ABSTRACT The interstitium of the heart muscle is primarily composed of ground substance. The glycoproteins and proteoglycans that formed the ground substance bore negative charges at neutral pH like the glycosaminoglycans and proteoglycans of the water-rich phase of the interstitium. Microelectrodes were used to look for the existence of an electrical potential between the interstitium of the rabbit papillary muscle and an ambient medium. Evidence is presented for the existence of such an electrical potential. When the ambient solution was a Krebs solution, this potential was evaluated at −5.7 mV. This electrical potential is dependent on the filling solution of the microelectrodes and on the ambient medium; in rabbit serum, the electrical potential diminished to −0.6 mV. Assuming that this potential is a measure of the Donnan potential, the Cl and Na activities in the interstitium were evaluated to 76 and 135 mM when the rabbit papillary muscle was superfused with a Kreb's solution.

INTRODUCTION

When a system contains nondiffusible electrolytes, these charged molecules introduce a gradient in the distribution of diffusible ions, and an electrical potential between this system and an ambient solution (1). The interstitium of the heart muscle is filled principally of ground substance (2) with blood vessels, the collagen and the connective tissue cells completing this extracellular space (2). With the isoelectric points of the collagen fibers between 7.0 and 5.0 and the low isoelectric points (pH 2.0 and 3.0) of the glycosaminoglycans and proteoglycans (3) at physiological pH, the interstitium contains fixed nondiffusible negative electrical charges. The presence of these fixed charges has been observed by the binding of La^{+++} (2) in the myocardial interstitium and more recently in skeletal muscles with polycationized ferritin (4).

The nondiffusible fixed charges put a constraint on the free diffusion of small ions between the vascular vessels and the interstitium. A comparison between two methods to obtain samples of the interstitium fluids indicates that the liquid-paraffin cavity method is more appropriate (5) in obtaining samples. This method indicates that the cations (K and Na) are more abundant than the anion (Cl) in the interstitium (5). The distribution of radioactive ions and neutral molecules is another indicator of the influence of the fixed charge on the concentration of small ions in the interstitium (6). The distribution of [^{35}S] sulfate and [^{3}H] sucrose in the skeletal and heart muscles of rats and toads in vivo, suggests that these fixed charges may cause exclusion or accumulation of anions in the interstitium depending on the type of muscle (6).

The fixed charges of the interstitium, if their density reaches a sufficient level, should manifest themselves by an electrical potential between the interstitium and either the vascular vessels or an ambient solution. Such electrical potentials, due to the fixed charges of the cytoplasmic proteins, were recorded on skinned muscle fibers (7, 8, 9). We looked with microelectrodes to determine if the density of the fixed charges in the interstitium is sufficent to induce a measurable electrical potential potential between the interstitium and the ambient solution. These measurements indicated that when a rabbit papillary muscle is superfused with a Krebs solution at 33°C, an electrical potential of −5.7 mV appears between the interstitium and the ambient solution.

METHODS

The experiments were performed on quiescent rabbit papillary muscles. Rabbits (1.5 kg) were stunned and the heart rapidly excised and placed in cold exygenated Krebs or in serum (4°C). Papillary muscles from the right ventricle were taken and transferred to a tissue bath (33°C) that contained either a Kreb's solution or a rabbit serum. The Kreb's solution contained in millimoles per liter: Na, 145; K, 5; Ca, 1.2; Cl, 127; HCO_3, 25; H_2PO_4, 1.2; and glucose, 5.5. For the purpose of studying the effect of NaCl concentration on the electrical potential between the interstitium and the ambient solution, NaCl concentration was reduced (to 30%) in the Kreb's solution and replaced by sucrose while the osmolarity of the solution was preserved. When oxygenated with 95:5% O_2/CO_2, the pH of either the Kreb's solution or the serum was between 7.35 and 7.45. The serum was prepared a few hours before the experiments. Samples of blood

(10 ml) were taken from rabbits, and after clotting at room temperature the samples were centrifuged at 3,000 *g* for 20 min.

The membrane potential and interstitium potential were measured with microelectrodes filled with 3 M KCl, 0.05 M NaCl, or Kreb's solution. The micropipettes were pulled from Pyrex glass (7740; Corning Medical, Glass Works, Medfield, MA), the outside surface of their tips covered with a hydrophobic material (Prosil-28, SCM Corporation, Gainesville, FL) and filled as described before (10). These microelectrodes had tip potentials <3 mV when plunged in a Kreb's solution.

The Cl liquid-ion exchanger microelectrodes were prepared as described before and had the same characteristics (10), except that the hydrophobic material was changed for Prosil-28 and the liquid-ion exchanger was a Corning liquid ion-exchanger (11) (No. 477913; Corning Medical, Corning Glass Works, Medfield, MA). This ion-exchanger presents the major advantage of a lower resistivity. The Cl microelectrode potential and the membrane potential were measured with an electrometer (model 602; Keithly Instruments, Inc., Cleveland, OH) (input impedance $>10^{14}$ Ω) and a Honeywell electronic recorder (Honeywell Information Systems, Inc., Waltham, MA). All the values reported are means, ±SE, unless mentioned otherwise. The data were tested for statistical significance with a *t* test ($P < 0.05$).

RESULTS

Electrical Potential between the Interstitium (V_{it}) and the Ambient Medium (Kreb's Solution)

A sample of the measurement recorded to look for V_{it} (interstitium potential, the difference of potential between the interstitium and the ambient solution measured in millivolts) with a microelectrode filled with a solution of KCl (3 M) is presented in Fig. 1. The microelectrode was used as for membrane potential measurements except that after being placed in a cell at the surface of the muscle it was pushed across the cell placing the microelectrode in the interstitium in order to detect the presence of an electrical potential between the interstitium and the ambient solution. Since the potential V_{it} was not influenced by the depth of the microelectrode impalement, Table I lists the values recorded no deeper than three cell layers from the endocardial surface. Fig. 2 is the histogram of the values observed for V_{it} when the ambient solution was the Kreb's solution. The measurement of a potential V_{it} necessitated a microelectrode filled with a 3 M KCl solution in order to reduce the liquid junction potential to a lower value that had little variation with different biological solutions. However, to verify the influence of the filling solution, the microelectrodes were filled with different solutions. First, the microelectrodes were filled with the Kreb's solution. This condition is particularly interesting because with the microelectrode being in the interstitium, the electrical potential V_{it} and the potential between the interstitium and the interior of the microelectrode are in opposition, and the potential should return to the potential measured in the Kreb's solution. An example of the measurement realized with a

TABLE I
INTERSTITIUM POTENTIAL (V_{it}) AND MEMBRANE POTENTIAL (V_m)

Conditions		V_{it}	V_m
Perfusion medium	Microelectrode-filling solutions		
		mV	
Krebs	3 M KCl	−6.3* ± 0.17 (177-12)	−77.3 ± 0.35 (181-12)
Krebs	Krebs	−1.3 ± 0.18 (53-5)	−58.5 ± 0.52 (53-5)
Krebs	0.05 M NaCl	+5.8* ± 0.81 (34-5)	−42.7 ± 0.72 (39-5)
Krebs 30% NaCl	3 M KCl	−12.4* ± 0.54 (54-5)	−78.0 ± 0.79 (59-5)
Serum	3 M KCl	−1.9* ± 0.27 (15-3)	−80.5 ± 0.6 (20-3)

Values are means ±SE number of measurements and number of muscles in parentheses.
*This value of V_{it} is statistically different ($P < 0.05$) from the value observed with Kreb's solution as the perfusion medium and the microelectrode-filling solutions.

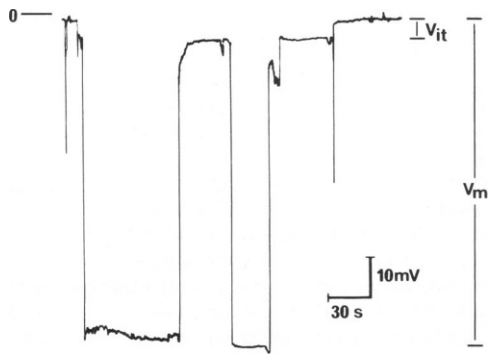

FIGURE 1 Example of the measurement of the interstitium potential, V_{it}, with the Kreb's solution as the ambient solution. The microelectrode was filled with KCl (3 M). V_m is the membrane potential.

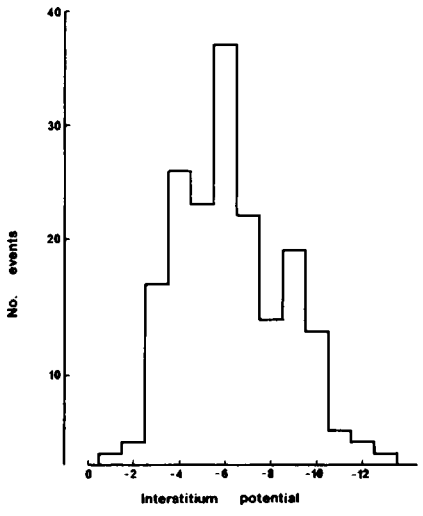

FIGURE 2 Histogram of the interstitium potential with the Kreb's solution as the ambient solution and the microelectrodes filled with KCl (3 M). *y* is the frequency per unit (*x*) of the interstitium potential (in millivolts).

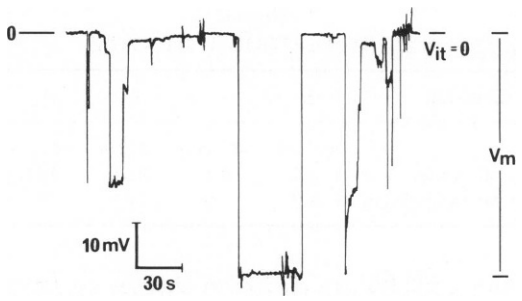

FIGURE 3 Example of the measurement of the interstitium potential, V_{it}, with the Kreb's solution as the ambient solution and the filling solution of the microelectrode. V_m is the membrane potential.

microelectrode filled with a Kreb's solution is presented in Fig. 3. A reduction of the membrane potential was observed with this filling solution and V_{it} was reduced to zero. Fig. 4 is the histogram of the values observed for V_{it} with a Kreb's solution in the microelectrode. Even if there is a maximum in this histogram for potential zero, there is a stemming of the values in negative values. When the filling of the microelectrode is a solution containing less NaCl than the Kreb's solution, a positive value for V_{it} should be observed. The mean value of V_{it} observed with this condition, using a Kreb's solution as the ambient solution and 0.05 M NaCl in the microelectrode, is presented in Table I, + 5.8 mV ± 0.81.

Electrical Potential in the Interstitium (V_{it}) with a Kreb's Solution with 30% NaCl, or a Serum as Ambient Solutions

If V_{it} is dependent on the filling solution of the microelectrode, it should also be dependent on the composition of the ambient solution. Two solutions were used: a Kreb's solution containing 30% NaCl and a serum freshly prepared before the measurements. The mean values of V_{it} observed when the ambient solution is a Kreb's solution with 30% NaCl or a serum are presented in Table I. These measurements are, respectively, −12.9 ± 0.54 and −1.9 ± 0.27 (Table I).

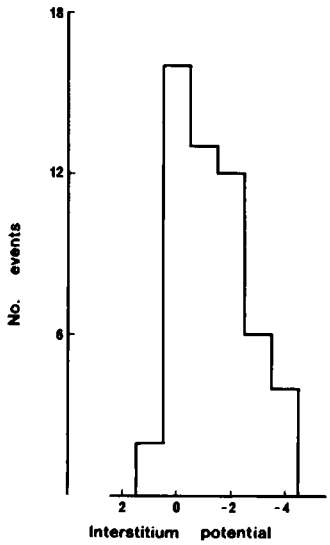

FIGURE 4 Histogram of the interstitium potential with the Kreb's solution as the ambient solution and the filling solution of the microelectrodes. y is the frequency per unit (x) of the interstitium potential (in millivolts).

Distribution of Cl between the Interstitium and the Ambient Solution

To verify if the Cl distribution, between the interstitium and the ambient solution was at equilibrium, impalements of cells and of the interstitium were done with Cl-selective microelectrodes. The differences of potential of the Cl-selective microelectrodes with the microelectrode in the interstitium and in the ambient solution are presented in Table II with the Kreb's solution and a fresh serum as ambient medium.

DISCUSSION

First, two important points related to the measurements of electrical potential with microelectrodes must be considered: (*a*) the response time of the microelectrodes and the liquid junction potential between the filling solution of the microelectrode; and (*b*) the electrolyte solution outside the microelectrode. For salt-bridge reference electrodes the response time is dependent on the geometry of the liquid junction (12) and is less than a second (90% response) for a thin hole or a cone, and a number of minutes for a cylinder. Since the geometry of the microelectrode at the tip is conical with a small angle (~10°); it is important to verify the response time of the microelectrode. When the microelectrodes were filled with a Kreb's solution, the response time was evaluated from the measurement of the potential that has an expected value of zero (confirmed for cation-exchange resin beads [3]) between the interstitium and the ambient solution. The response times were <10 s with microelectrodes filled with a Kreb's solution. A sample of these measurements is shown in Fig. 2. Since the concentrations of Na^+ and Cl^- may be different for the interstitium and the ambient solution, it may introduce a change of the liquid junction potential at the open tip of the

TABLE II
CL-MICROELECTRODE POTENTIALS ($\Delta V_{Cl,i}$)

Perfusion solution	$\Delta V_{Cl,i}$
	mV
Krebs	−0.95 ± 0.20 (30-3)
Serum	−0.23 ± 0.17 (13-3)

Values are means ±SE number of measurements and number of muscles in parentheses. $\Delta V_{Cl,i}$ is the difference of potential (in millivolts) between the Cl microelectrode potential in the interstitium and that in an ambient solution.

microelectrode. An attempt (Appendix) to evaluate this liquid junction potential indicated a change of 0.7 mV between these two conditions since in both mediums the interstitium and the ambient solution, Na^+ and Cl^- are the major electrolytes.

The histograms of V_{it} measured with microelectrodes filled with either a 3 M KCl or a Kreb's solution both presented an asymmetrical distribution, although the asymmetry is more evident for microelectrodes filled with a Kreb's solution. This may have arisen from an increase of the electrical resistance at the open end of the microelectrode by the molecules of the ground substance. Such an increase, as observed (14) for an open tip microelectrode, increases the tip potential of the microelectrode. When the filling solution of the microelectrode contained 0.05 M NaCl, the potential V_{it} was positive. This positive value was expected from the liquid junction potential as calculated for a junction with fixed charges (15).

The membrane potential was dependent on the filling solution of the microelectrode. This resulted from a change in the junction potential between the microelectrode and the myoplasm (16) when the filling solution of the microelectrode, 3 M KCl, was replaced by a Kreb's solution. The influence of the microelectrode filling solution on the membrane potential is similar to the observations made on frog sartorius muscle cells (16).

The difference in the Cl microelectrode potential when the microelectrode was in the interstitum and the Kreb's solution was zero. This indicates that the distribution of Cl is at the equilibrium. The same conclusion is valid when the ambient solution was replaced by a rabbit serum.

Assuming that the influence of the substances and molecules that filled the interstitium is the same on microelectrodes filled with either a Kreb's solution and/or a 3 M KCl solution, then the difference of electrical potential between the interstitium and the ambient solution was evaluated to -5.7 mV when the papillary muscle is superfused with a Kreb's solution, this value included a correction for a change in the liquid junction potential between the Kreb's solution and the interstitium (Appendix). A significant contribution of diffusion potentials to V_{it}, as observed on skinned muscle fibers with ATP (17), was eliminated considering the distribution of Cl observed with Cl microelectrode. The nonpermeable electrical charges of the ground substance or of the macromolecules confined to the interstitium by the endocardium introduce a Donnan distribution between the interstitium and the ambient solution for the permeable ions, and assuming that V_{it} is a measure of the Donnan potential, the Cl and Na activities in the interstitium were evaluated to 76 and 135 mM/l, with the Kreb's solution as the ambient solution.

When the Kreb's solution is replaced by a serum, then the value of V_{it} is reduced almost to zero because of the presence of molecular anionic residues. Although the serum contains many substances and molecules with different distribution ratios, it does not necessarily mean that the serum contains as many fixed charges as the interstitium. This result supports the observation that in vivo, there are no differences between the sucrose and $SO_4^=$ spaces in rat ventricles (6).

TABLE III
LIQUID JUNCTION POTENTIAL

Junction	U_1	V_1	U_2	V_2	V_L
	mΩ/cm	*mΩ/cm*	*mΩ/cm*	*mΩ/cm*	*mV*
KCl (3 M)-Krebs	6.7	9.5	164	174	1.79
KCl (3 M)-interstitium	8.0	7.9	164	174	2.45

APPENDIX

Liquid Junction Potential

The liquid junction potential in millivolts (V_j) between a solution of KCl (3 M) and the Kreb's solution or the interstitium was estimated by the Henderson's equation I-1;

$$V_j = \frac{RT}{F} \frac{(U_1 - V_1) - (U_2 - V_2)}{(U_1 + V_1) + (U_2 + V_2)} \ln \frac{U_1 + V_1}{U_2 + V_2}. \quad \text{(A1)}$$

U and V are the conductances of the cations and anions for both solutions, R, T, and F have the conventional meanings. The values of V_j, U, and V are summarized in Table III. (18).

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