Christopher J. Hyatt, John J. Lemasters, Barbara J. Muller-Borer, Timothy A. Johnson and Wayne E. Cascio


You might find this additional information useful...

This article cites 19 articles, 13 of which you can access free at:

http://ajpheart.physiology.org/cgi/content/full/274/6/H2001#BIBL

This article has been cited by 4 other HighWire hosted articles:

Region of slowed conduction acts as core for spiral wave reentry in cardiac cell monolayers
[Abstract] [Full Text] [PDF]

Novel method for measuring junctional proton permeation in isolated ventricular myocyte cell pairs
P. Swietach and R. D. Vaughan-Jones
[Abstract] [Full Text] [PDF]

Coverlip hypoxia: a novel method for studying cardiac myocyte hypoxia and ischemia in vitro
K. R. Pitts and C. F. Toombs
[Abstract] [Full Text] [PDF]

Localized injury in cardiomyocyte network: a new experimental model of ischemia-reperfusion arrhythmias
A. Arutunyan, D. R. Webster, L. M. Swift and N. Sarvazyan
[Abstract] [Full Text] [PDF]

Medline items on this article's topics can be found at http://highwire.stanford.edu/lists/artbytopic.dtl on the following topics:

- Medicine .. Myocardium
- Medicine .. Microscopy
- Chemistry .. Viscosity
- Physiology .. Rats

Updated information and services including high-resolution figures, can be found at:

http://ajpheart.physiology.org/cgi/content/full/274/6/H2001

Additional material and information about AJP - Heart and Circulatory Physiology can be found at:

http://www.the-aps.org/publications/ajpheart

This information is current as of March 20, 2008.
A superfusion system to study border zones in confluent cultures of neonatal rat heart cells

CHRISTOPHER J. HYATT, JOHN J. LEMASTERS, BARBARA J. MULLER-BORER, TIMOTHY A. JOHNSON, AND WAYNE E. CASCIO

1Department of Biomedical Engineering and Division of Cardiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599
2Department of Medicine, and 3Department of Cell Biology and Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Hyatt, Christopher J., John J. Lemasters, Barbara J. Muller-Borer, Timothy A. Johnson, and Wayne E. Cascio. A superfusion system to study border zones in confluent cultures of neonatal rat heart cells. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H2001–H2008, 1998.—We present a new experimental method to study intracellular ion regulation in cultured cardiomyocytes at a border zone separating two different and distinct environments. Our system uses a dual-flow superfusion chamber to produce two different but adjacent environments over a monolayer of cardiomyocytes. Fluorescent microscopy of fluorescein showed that the transition between the two environments was nearly linear and was 220–320 µm wide depending on fluid viscosity and velocity. We superfused cultured monolayers on one side with a solution at pH 6.5 and on the other side with a solution at pH 7.4. We observed a sharply demarcated difference in intracellular pH (pHi) between the two halves of the cell monolayer as measured with the fluorescent pH i indicator carboxy-seminaphthorhodafluor-1. The demarcation of pHi corresponded well with the demarcation of the border measured with fluorescein. We conclude that our superfusion system will facilitate the study of intercellular communication and interactions across boundaries of cardiac tissue where different ionic or metabolic conditions are present, for example, between ischemic and nonischemic myocardium.

intracellular pH; gap junctions; ischemia; arrhythmia; monolayer

EXISTING EXPERIMENTAL models for studying systems of cardiac myocytes typically involve examination of intact myocardium such as whole heart preparations, isolated papillary muscles, and ventricular trabeculae. These models have frequently been used to study myocytes at a “border zone,” a region of myocardial tissue in which normal myocytes are juxtaposed with myocytes with altered ionic, metabolic, and electrical characteristics, usually because of ischemic or hypoxic stress (4, 9–11, 20). Existing models do not allow easy examination of intracellular changes in myocytes; however, nor do they allow the direct observation of gap junctional communication between myocytes.

Confluent monolayers of cultured cardiac myocytes are used to study impulse propagation (7, 8, 15–17) and communication between myocytes through gap junctions (12). Confluent culture models provide the unique opportunity to study intracellular changes and intercellular interactions in considerably greater detail than models based on intact tissue. In a culture model, tissue geometry is greatly simplified, signals from single cells can be identified and measured, and the extracellular environment of the myocytes can be easily manipulated. Furthermore, heart cell culture models have been improved by approximations to in vivo tissue morphology through the use of both anisotropic monolayers of cultured myocytes cultured on a growth-directing collagen substrate (8) and patterned growth of cultured myocytes (17). However, no methods exist to produce border zones in confluent cultures of cardiac myocytes where cells in a normal environment are placed directly adjacent to myocytes in an altered environment.

We introduce in this paper a novel method that uses confluent cultures of neonatal rat cardiomyocytes to study myocyte interactions at the border of two different cellular environments. Our model should contribute significantly to the understanding of intracellular changes in myocytes and gap junctional communication between myocytes at a border zone.

MATERIALS AND METHODS

Cell Preparation

We prepared confluent monolayers of cultured neonatal rat cardiac myocytes essentially as described by Rohr and Salzberg (16). In brief, we anesthetized 8–12 neonatal rats (Sprague-Dawley, Charles River Laboratories) with isoflurane (Isoflor, Barber Vet Supply, Raleigh, NC), removed the hearts, and put them on ice in a solution of Hanks' balanced salts solution without Ca²⁺ and Mg²⁺ (GIBCO-BRL, Grand Island, NY). We retained the lower two-thirds of the ventricles, minced them into pieces ~1 mm in size, and transferred the tissue into a dissociating solution consisting of Hanks' balanced salt solution without Ca²⁺ and Mg²⁺ (GIBCO-BRL), 0.1% trypsin (GIBCO-BRL), 60 µg/ml pancreatin, 20 µg/ml streptomyccin, 20 U/ml penicillin, and 50 ng/ml amphotericin B (Sigma Chemical, St. Louis, MO). We gently stirred the tissue suspension at 37°C for 12-min duration and discarded the supernatant after settling of the tissue fragments. We then added fresh dissociating solution and resumed stirring. After each subsequent stirring, we allowed the tissue fragments to settle, retained the supernatant with dissociated cells, and added the supernatant into centrifuge tubes. To terminate further digestion of the cells in the centrifuge tubes, we added culture media consisting of medium 199 with Hanks' salts (GIBCO-BRL), 10% newborn calf serum, 1.5 µM vitamin B₁₂, 20 µg/ml streptomyccin, 20 U/ml penicillin, and 50 ng/ml amphotericin B. We repeated this cycle of stirring and supernatant collection until the remaining tissue was almost completely dissociated. Complete dissociation usually occurred after three to four stirring cycles of 12 min each. We then centrifuged the supernatants for 3 min at 1,000 revolutions/min. We resuspended the resulting cell pellets in culture medium and transferred to an incubator (at 37°C, 1.2% CO₂). After prepping the cell suspension in a culture flask for 2 h to remove fibroblasts and endothelial cells, we plated.
the cell suspension on 40-mm glass coverslips (no. 1 thickness, Bioptechs, Butler, PA) coated with collagen type IV from human placenta (Sigma). We seeded the coverslips at a density of 1,500–2,000 cells/mm². We exchanged cell media everyday thereafter with the culture media described above except with 5% newborn calf serum.

Dual-Flow Superfusion Chamber

Successful separation of two regions of flow depended in part on the design of the silicone rubber gasket used to create the cavity in the chamber through which solutions flow (see Fig. 1). We used a gasket containing two channels originating from two inlet ports that gradually increased in width until approximately one-third of the total distance across the chamber. At this point, the flows were brought together nearly parallel to one another. After traversing the open middle of the chamber cavity, the flows entered two channels that gradually decreased in width until the outlet ports were reached. The depth of the cavity was 1.0 mm, and the total volume of the cavity was ~0.67 ml.

We placed the silicone rubber gasket on a round (40-mm diam) Plexiglas plate with four holes through which steel tubing delivered the superfusate solutions to the cavity (see Fig. 2). We placed the stainless steel tubing (18-gauge perfusion set) in place with a Teflon ring mounted below the Plexiglas plate. We held the stainless steel tubing (18-gauge perfusion set) in place with a Teflon ring mounted below the Plexiglas plate. We placed the glass coverslip (40-mm diam, no. 1 thickness) with cultured cardiomyocytes on the gasket with the myocyte monolayer facing down into the cavity. We began superfusion immediately to keep the cells moist throughout the mounting procedure. To protect the coverslip, we placed a silicone rubber washer on top of the coverslip and clamped the whole apparatus together through use of upper and lower aluminum clamps that were gently brought together using thumb screws. We applied equal turn tightness to each thumb screw to ensure even pressure on the coverslip.

Pump System

We integrated the superfusion chamber into a system for optical epifluorescence measurements as depicted in Fig. 3. We pumped solutions into and out of the superfusion chamber using a peristaltic pump equipped with a reduced pulsation, eight-roller pump head (Cole-Parmer). In addition to having eight rollers instead of the usual three or four rollers, the pump head was equipped with a two-cartridge system designed to further reduce pulsations (Cole-Parmer). The eight-roller pump head was also designed for very low flow rates and thus was an ideal choice for our system. We considered other pump systems, including syringe pumps, but no other pump system offered the same ease of use and ability to achieve low flow rates. We included pulse dampeners (Cole-Parmer) in-line to eliminate residual pulsations on both the inlet and outlet sides of the system. The pulse dampeners had a dead volume of 190 ml. We used Norprene food tubing (L/S size 14, Cole-Parmer) in the peristaltic pump head and Tygon tubing (L/S size 14, Cole-Parmer) elsewhere. We selected one of three solutions for superfusion on one side by appropriate opening and closing of a five-valve system. When a solution was not being used, it could either be pumped back to its source or sent to waste. We typically included the water-soluble fluorescent dye fluorescein in the unswitched control line for continuous monitoring of the boundary between the superfusates.

We attached the superfusion chamber to an X-Y table (Parker Hannafin, Daedal Division, Harrison City, PA) with a movement of 10-µm resolution. The X-Y table with attached superfusion chamber was in turn mounted onto a custom stage attached to the microscope.

Finite Element Analysis

Finite element analysis was used to determine flow characteristics that could not be easily determined experimentally, such as flow velocity within the cavity of the chamber and shear stress at the coverslip surface. The finite element model was developed using Fluent software ( Fluent, Lebanon, NH). The finite element grid of the cavity created by the gasket consisted of $44 \times 26 \times 10$ or $11,440$ cells. The inlets and outlets were modeled as square ports $1.0 \text{ mm}^2$ in area with flow entering parallel to the top and bottom of the cavity. This is an approximation to the inlets and outlets of the actual chamber, which were placed perpendicular to the top and bottom of the cavity. Flow entering the cavity perpendicu-
larly, however, quickly became parallel during the initial millimeters of entrance length into the cavity and could be modeled with a fair degree of accuracy as entering the cavity parallel to the top and bottom plate.

Inlet and outlet fluid flow velocities were set as boundary conditions at three of the four ports and were set to an identical magnitude. The fourth port (an outlet) was modeled to accept any remaining flow so that conservation of mass was obeyed and thus the fourth port had a flow rate exactly matching the other three ports. Laminar (nonturbulent) flow was assumed throughout the cavity at all flow rates.

### Fluorescent Intracellular pH Indicator Dye

Intracellular changes in pH in cultured myocytes were estimated using the fluorescent pH indicator carboxy-seminaphthorhodafluor-1 (SNARF-1) (Molecular Probes, Eugene, OR). Carboxy-SNARF-1 undergoes a shift in the relative intensity of two emission peaks, 640 nm and 585 nm, upon a change in pH. We obtained estimates of intracellular pH by calculating the ratio of intensities at the two emission wavelengths (5). For loading, myocytes were incubated with 10 µM carboxy-SNARF-1-acetoxymethyl ester (AM) for 40 min at 37°C in 5% CO₂ with air. We prepared the dye solution by dissolving 50 µg carboxy-SNARF-1-AM in 200 µl DMSO (Sigma) followed by vigorous mixing into HEPES-buffered medium 199 solution (GIBCO-BRL) with 5% newborn calf serum (GIBCO-BRL) to obtain a final concentration of 10 µM.

We typically acquired carboxy-SNARF-1 fluorescence images at 585 nm for 10 s and at 640 nm for 2 s, but the exposure time was varied as necessary to obtain images with adequate intensity. We computed the intensity ratio on a pixel-by-pixel basis.

We performed an approximate calibration to convert SNARF-1 ratio values to intracellular pH values as follows. We prepared three HEPES-buffered saline solutions with 50 mM carboxy-SNARF-1. The three solutions were titrated to three different pH values: 6.8, 7.2, and 7.5. We performed confocal epifluorescence imaging of the three solutions to obtain SNARF-1 fluorescence ratio values as a function of pH. Furthermore, we assumed an intracellular pH of 7.3 for the myocytes during control superfusion. We based this assumption on values found by ourselves and others for physiologically normal intracellular pH in cultured cardiomyocytes (3, 5). The calibration curve was thus adjusted upward or downward to ensure an intracellular pH of 7.3 during control superfusion. Therefore, the values of intracellular pH we obtained can only be considered approximate, but we expect that the results we obtained are qualitatively accurate.

### Imaging System

We used an upright microscope (Nikon, Melville, NY) in bright-field mode to perform the fluorescence imaging. The microscope was equipped with a 100-W mercury arclamp and was used in conjunction with a 546-nm excitation filter and 585 ± 10-nm bandpass and 620-nm long-pass emission filters (Omega Optical, Brattleboro, VT). We used Nikon objectives of ×4 and ×10 power. The emitted fluorescence was focused onto a digital charged couple device camera (TE/CCD-1317-K, Princeton Instruments, Trenton, NJ) under software control. We used Metafluor software (Universal Imaging, West Chester, PA) running on a 486-DX 33-MHz computer to control the microscope imaging system for acquisition of images.
Simultaneous Use of Two Dyes

The water-soluble sodium salt of fluorescein (Sigma) may be used in conjunction with carboxy-SNARF-1-AM for simultaneous imaging of the demarcation of the border zone and changes in SNARF-1-AM ratio fluorescence due to intracellular pH changes. The water-soluble salt of fluorescein does not readily cross cell membranes and thus is unlikely to affect cell function or intracellular pH. Both dyes may be used simultaneously, because their excitation and emission spectral characteristics are sufficiently different to allow separate resolution of each dye. The spectral properties of fluorescein are such that its wavelengths of peak excitation and emission (494 and 520 nm, respectively) are much shorter than the wavelengths of peak excitation and emission of carboxy-SNARF-1-AM (single 534-nm excitation and dual peaks of 580- and 640-nm emission).

As long as fluorescein is at sufficiently low concentrations (typically 1 µM or less), it can be imaged simultaneously with SNARF-1-AM without any spectral interference between the two dyes. When we imaged the superfusate border at SNARF-1 wavelengths, we could observe no fluorescence due to fluorescein (at 1 µM concentration).

Extracellular pH Gradient Across the Border

We formed a gradient in extracellular pH at the border zone (i.e., centerline of the chamber) by superfusion on one side using Hanks' balanced salt solution buffered with 10 mM HEPES to pH 7.4, and on the other side with Hanks' balanced salt solution buffered with 10 mM PIPES to pH 6.5. The Hanks' balanced salt solution (GIBCO-BRL) contained (in mM) 137.0 NaCl, 5.4 KCl, 1.3 CaCl₂, 0.8 MgSO₄, 4.2 NaHCO₃, 0.5 KH₂PO₄, 0.3 Na₂HPO₄, 5.0 glucose, and 10.0 HEPES or PIPES. NaOH was used for final adjustment of pH. Experiments were carried out at room temperature (22°C).

RESULTS

Finite Element Model

Streaklines. Figure 4 shows streaklines in a finite element analysis of the fluid-flow characteristics of the superfusion chamber. Streaklines represent the path taken by a stream of particles placed at a given point of origin in the fluid and are equivalent to the streams of injected dye commonly used in experimental fluid models to analyze flow patterns.

The origins of seven streaklines were placed in front of each inlet at the midpoint between the top and bottom plate, and the resulting trace or “streakline” showed the path the particle streams took across the volume of the cavity to the corresponding outlet. At all flow rates studied, the streaklines were evenly spaced and nearly parallel to one another across the centerline (border zone) of the cavity.

Velocity profile along the chamber depth. Finite element modeling indicated that the flow was “fully developed” at the point at which the two separate flows met. A fully developed flow is one in which the velocity profile of the flow along the depth of the cavity is no longer changing in the direction of the flow (14). In contrast, flow near the inlets could be expected to be “developing.” A fully developed flow driven between two stationary flat walls (which are the upper and lower plates of the chamber) by an externally imposed pressure gradient is termed plane Poiseuille flow (14). In plane Poiseuille flow, parabolic velocity profiles exist along the depth of the cavity. Flow velocity is zero at both the upper plate (coverslip) and lower plate (Plexiglas) and reaches a maximum at the midpoint between the upper and lower plates.

Shear stress. We also estimated shear stress at the coverslip surface using the finite element model. Shear stress in plane Poiseuille flow is greatest at the walls and decreases linearly until the midpoint between the walls is reached, at which point zero stress is attained. The shear stress is thus greatest at the coverslip surface. Values calculated from the finite element model for the coverslip shear stress at the centerline of the cavity were 0.7–4.2 mdyne/cm² at flow rates ranging from 0.5 to 3.0 ml·min⁻¹·inlet⁻¹.

Flow velocity. The finite element analysis revealed that flow velocities were quite uniform away from the inlets and outlets, particularly along the centerline of the chamber where cellular measurements were made. At a flow rate of 1.5 ml·min⁻¹·inlet⁻¹, the velocity (parabolic maximum) was ~3.0 mm/s along most of the centerline of the chamber. Because the velocity profile is parabolic, the average velocity along the depth of the cavity at 1.5 ml/min flow rate per inlet would be ~2.0 mm/s. It is interesting to note that the average velocity of 2.0 mm/s in our flow chamber is on the order of measurements of the average velocity of blood flow in capillaries, which has been estimated to be 1.0 mm/s (2).

Experiments

Measurements of border zone width. We performed measurements of the width of the transition zone or border zone measured perpendicular to the direction of flow from one superfusate flow to the other superfusate flow at the centerline of the chamber. To measure the degree of separation of the side-by-side flow of the two superfusates, we added 1 µM fluorescein to the solution superfusing one side of the cell monolayer and added no dye to the solution superfusing the other side of the cell monolayer (see Fig. 5A).

Epifluorescent measurements revealed a sharp transition from one superfusate to the other. A line scan...
measured perpendicularly across the border zone using ImageTool 1.23 software (Univ. of Texas Health Science Center at San Antonio) showed a sigmoidal shaped intensity profile (Fig. 5B). We fit this sigmoidal intensity profile to an empirical equation

\[ I(x) = \frac{A}{1 + \exp[B(x - C)]} + D \]  

where \( I(x) \) is the intensity at distance \( x \) (or pixel \( x \)). We fit the parameters \( A, B, C, \) and \( D \) to the linescan data using SigmaPlot 2.0 software (Jandel Scientific, San Rafael, CA). We defined the border width as the difference between the points at which the intensity was 5 and 95% of the total fluorescence increase (parameter \( A \)) above the lowest intensity level (parameter \( D \)) [i.e., \( D + 0.05(A - D) \) and \( D + 0.95(A - D) \)]. When defined in this manner, the transition region measured \( \sim 270 \mu m \) wide.

The width of transition region depended on several factors, however, including flow velocity and fluid viscosity. By varying the flow velocity from 0.5 to 3.0 ml·min\(^{-1}\)-inlet\(^{-1}\), the width of the transition region between the two flows could be controlled by from \( \sim 320 \) to \( 220 \mu m \) (Fig. 6). The width of the transition region was also affected by solutions of varying viscosity. Results of preliminary experiments using solutions with dissolved dextran (0–40 g/l) to adjust the viscosity of the solution showed that the border width could be increased by \( \sim 20\,\text{--}\,25\% \) with highly viscous solutions (>20 g/l). We found, however, that fine control of border width was much more difficult to achieve by altering fluid viscosity than by altering flow rate.

Measurements of intracellular pH. We performed experiments where one-half of a confluent monolayer of cardiomyocytes was superfused with solution at pH 6.5 while the other half was superfused with a solution at pH 7.4. We determined intracellular pH by ratiometric measurement of carboxy-SNARF-1 fluorescence. Figure 7 shows intracellular pH at the border between the pH 6.5 superfusate and the pH 7.4 superfusate after 0, 1, 2, and 6 min of superfusion. The transition from cardiac myocytes with an acidic intracellular environment to myocytes with a normal pH closely corresponded to the sharp superfusion gradient in fluorescein epifluorescence. After 2 min (Fig. 7C), large decreases in intracellular pH occurred on the acidic superfused side. After 6 min of acidic superfusion, further intracellular acidification occurred in cardiomyocytes in the acidic superfused side. Myocytes deep into the acidic zone reached an intracellular pH of 6.9–7.0 (Fig. 7D).

**DISCUSSION**

**Finite Element Modeling**

Using a finite element model, we were able to estimate flow velocity and shear stress at the coverslip surface, parameters which would have been difficult to measure experimentally because our flow chamber was a closed system. Low flow rates ensured that the cultured myocytes were exposed to minimal shear forces, preventing cell detachment. Shear stresses in our superfusion system were on order of 0.001 dyn/cm\(^2\).
and were considerably less than shear stresses measured in superfusion systems developed for studying the effects of shear stress on cultured endothelial cells. The shear stresses in these superfusion systems ranged from 1 dyn/cm² to as much as 120 dyn/cm² (13, 19). Thus the low shear stresses that occurred in our system would be unlikely to have an adverse effect on cultured myocytes, even during experiments of long duration. Shear rates near the inlets and outlets were relatively high when compared with the center, but these regions were not examined and should not influence the behavior of cardiac myocytes near the center of the coverslip where epifluorescent measurements of intracellular pH and border width (fluorescein measurements) were made.

Finite element modeling will likely prove useful in future studies of the effect of flow rate and flow patterns on the myocyte monolayer and their effect on the width of the border zone. Different flow rates are likely to affect the diffusion of substances in the layer of superfusing fluid, and information obtained from the finite element model about the flow velocity at the superfusion border zone will be important in determination of the effect of flow rate on diffusion of these substances in the border region.

**Border Zone Width**

Results of experiments using fluorescein to measure the width of the transition region between two parallel flows showed conclusively that it is possible to create a well-demarcated border zone in confluent monolayers of neonatal rat heart cells. These findings were underscored by the results of experiments using carboxy-SNARF-1 to measure intracellular pH during side-by-side superfusion with acidic and normal solutions.

The experiments using fluorescein dye showed that the transition region from one flow to the other flow across the centerline of the chamber was 220–320 µm in width. The width of the border zone also depends on other factors, such as the diffusion characteristics of the substance dissolved in solution, medium viscosity, and the temperature of the solution. It is likely that ions, gases (e.g., O₂ and CO₂), and other solutes, when dissolved in one superfusate but not the other, will produce different gradients across the border because of differences in their diffusion properties. These initial experiments with fluorescein, however, have provided a reference for further tests of border zone width.

**Intracellular pH Measurements**

Our experiments showed that intracellular pH fell rapidly on the acidic half of the monolayer during side-by-side superfusion with acidic solution and normal solution. A decrease in intracellular pH occurred within 1 min after the start of acidic superfusion. The width of the intracellular pH transition region was comparable to the superfusate transition region measured using fluorescein dye. A change from intracellular pH from 7.3 to ~7.0 occurred gradually over a distance of ~250 µm. This distance was similar to the spatial gradient measured using fluorescein dye (compare Fig. 7 with Fig. 5).

**Relevance of Our Model to Border Zones In Vivo**

We should note that our model cannot simulate a true ischemic border, since there is continual washout of extracellular environment. Thus extracellular metabolites, ions, and gases that typically accumulate during ischemia (e.g., lactate, K⁺, and CO₂) do not accumulate in the extracellular space of the cultured cell monolayer. The strength of our model, however, resides in its ability to simulate the intracellular conditions that exist at the border during an ischemic event. We can then examine how gradients in intracellular ions and metabolites impact on intracellular...
metabolism, cell-to-cell communication, electrical coupling, and impulse propagation at the border. It is well established that heterogeneity in extracellular ions, such as $K^+$ and $pH$, during ischemia contribute to altered electrophysiological properties that are arrhythmogenic. The role of intracellular ionic and metabolic changes in arrhythmogenesis remains unclear, particularly changes in intracellular pH and $Ca^{2+}$. Changes in these intracellular ions and metabolites are thought to contribute to cellular uncoupling and triggered arrhythmias (1, 6, 18, 22). It has hitherto been difficult to study these intracellular ions and metabolites in models of ischemia that utilize whole hearts or intact tissue specimens. Our model will make it possible to more easily study these intracellular changes and determine how they relate to changes in cell-to-cell communication, uncoupling, and injury currents at the border zone. It should be noted that the dimensions of the border width in our model (200–300 µm) contrast with early findings that the width of border zones in ischemic whole hearts of pig and dog have been estimated to be on the order of 1 cm. Wit and J anse (21), however, cite research that has shown that the transition from normoxic to hypoxic tissue is very sharp and well demarcated, whereas much more gradual gradients exist in extracellular $K^+$ and pH. In particular, the border zone of an ischemic region is characterized by a sudden and sharp transition from normal oxygen pressure to a very low oxygen pressure (from the normal to the ischemic zone), whereas extracellular $K^+$ and pH remain near normal levels in the vicinity of the border zone. Our model of the border zone has demonstrated the potential to accurately recreate similar conditions.

Additionally, recent research has shown that the extent of the extracellular gradient in pH and $K^+$ at the ischemic border zone can be much less than 1 cm. Wilensky et al. (20), for example, describe a model of the border zone in which coronary perfusion of the subendocardium of a rabbit septum was arrested but in which the endocardial surface continued to be superfused with oxygenated solution. They measured a border zone ~600 µm deep in which existed metabolic and ionic gradients in pH, phosphocreatine, and extracellular potassium. The transition region of our border zone can be made quite sharp, with border width values of 100 µm or narrower obtainable with high superfusion rates. Conversely, we can make the border region fairly wide by using viscous solutions and low superfusion rates, up to perhaps 500 µm or more, while maintaining a stable border position. So it is possible to recreate a variety of border widths in our model. Also, given that our model utilizes single layers of cultured myocytes as opposed to thick biological specimens, our border widths should be appropriately narrower to be physiologically realistic.

In conclusion, we have presented a methodology for dual-flow superfusion of monolayers of cultured neonatal rat cardiac myocytes for the purpose of creating sharp border zones. Our dual-flow superfusion chamber will allow the study of well-defined boundaries across which cardiac myocytes with differing environments can interact. Using this model, we are evaluating the importance of ionic and electrical interactions across boundaries of tissue regions with differing environments for the origin of arrhythmias. This model will also provide a geometry to compare experimental findings with mathematical simulations of impulse propagation and arrhythmogenesis across hypoxic and ionic boundaries.

We thank Dr. Yousef Sharief, Dr. Barry Pallotta, and Darrell Sandiford at the University of North Carolina at Chapel Hill and Dr. Stephanie Rohr, Dr. Andrè Kleber, and Regina Flückiger at the University of Bern, Switzerland, for advice and technical support.

This work was supported by National Heart, Lung, and Blood Institute Grant P01-HL-27430 and by Office of Naval Research Grant N00014–96–0283. C. J. Hyatt was supported in part by a University of North Carolina Board of Governors’ Fellowship in Science and Technology in the Department of Biomedical Engineering and in part by an American Heart Associate (North Carolina affiliate) Predoctoral Fellowship.

Portions of this work were presented at the 1996 Annual Fall Meeting of the Biomedical Engineering Society in State College, PA, October 3–6, 1996, and at the 12th International Conference of the Cardiovascular System Dynamics Society in Chapel Hill, NC, November 7–10, 1996.

Address for reprint requests: W. E. Cascio, Univ. of North Carolina School of Medicine, Campus Box 7075, Burnett-Womack Bldg., Rm. 346, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7075.

Received 27 August 1997; accepted in final form 16 February 1998.

REFERENCES


