Multitrack system for superfusing isolated cardiac myocytes

Lois Jane Heller,1 David E. Mohrman,1 Juline A. Smith,1 and Kendall B. Wallace2

1Department of Physiology and 2Department of Biochemistry and Molecular Biology, School of Medicine, University of Minnesota, Duluth, Minnesota 55812

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Heller, Lois Jane, David E. Mohrman, Juline A. Smith, and Kendall B. Wallace. Multitrack system for superfusing isolated cardiac myocytes. Am J Physiol Heart Circ Physiol 284: H1872–H1878, 2003.—A new system for studying mechanical activity of freshly isolated cardiac myocytes from up to four experimental groups simultaneously is described. Suspensions of cardiac myocytes isolated from adult rat hearts were drawn into microhemocrit capillary tubes, which were then mounted in parallel fashion between two four-channel tubing manifolds placed on the movable stage of an inverted microscope. Within a few minutes, cells settled and attached to the bottom of the tubes and then could be superfused with various test solutions. The system allowed for electrical field stimulation, rapid changes in bathing solutions, control of temperature, and simulation of ischemia and reperfusion with measurements of the effects of such interventions on both populations of cells (low power survey) and individual myocytes (high power). Myocyte responses to these various interventions are described. The primary advantage of this system is the ability to conduct experiments on cardiac myocytes isolated concurrently from multiple experimental groups at the same time and under identical conditions.

METHODS

Cardiac myocyte preparations. Adult male Harlan Sprague-Dawley rats (body weights = 373 ± 5 g) were housed in the University’s climate-controlled American Association for Accreditation of Laboratory Animal Care-accredited animal care facility on a 12-h:12-h light-dark cycle with free access to food and water. On the day of the experiment, rats were injected with heparin (1.0 IU/g body wt ip) 30 min before being euthanized with CO2. Hearts were rapidly removed from the thorax, placed in a preweighed beaker containing iced perfusate (Joklik’s calcium-free modified minimal essential media containing 0.1% BSA), and then weighed with aortic stump attached (weights = 1.55 ± 0.03 g). Cardiac myocytes were isolated from the rat hearts using standard enzymatic dispersal techniques (3–5, 17). Briefly, hearts were cannulated via the aorta for Langendorff-type constant pressure (40 mmHg) perfusion at 37°C with the perfusate passing through a gas-exchange column to equilibrate with 100% O2. After 5–10 min of nonrecirculating perfusion to wash blood from the coronary vasculature, the perfusate was switched to one containing 0.7% (200 U/ml) collagenase (Worthington), and perfusion was continued in a recirculating mode (with ~60 ml of solution) until the vascular bed deteriorated as indicated by a doubling of coronary flow (usually achieved after 20–30 min).

Hearts were then removed from the perfusion apparatus, and the tissue was minced and suspended in a beaker containing ~20 ml of fresh collagenase-containing solution. Digestion was continued in the beaker placed in a gyrating water bath at 37°C for an additional ~20 min with CaCl2 added after 10 min to a concentration of 50 μM. The tissue digestate was then slowly triturated through a wide-tipped pipette several times to help separate the cells and filtered through cheese cloth to remove nondigested material, and the cells were allowed to settle out of the filtrate. After removal of the supernatant, the cell preparation was resuspended at room temperature and resuspended in collagenase-free, calcium-free Joklik’s solution containing 1.5% BSA. This step was repeated twice. Initial yield of myocytes was assessed at this point by determining the number of rod-

THE ABILITY to study the contractile activity of isolated cardiac myocytes under a variety of experimental conditions has led to significant understanding of myocyte function. However, with the use of the standard approaches, it has not been possible to conduct simultaneous experiments on more than one preparation of isolated cardiac myocytes at a time. In addition, it has been necessary to use separate specialized apparatus for studying different aspects of myocyte behavior such as responses to electrical field stimulation, rapid changes in bathing solution composition, simulated ischemia, and reperfusion. We have developed a simple experimental system for subjecting isolated cardiac myocytes from up to four experimental groups simultaneously to identical treatments. This method allows for electrical field stimulation, rapid changes in bathing solutions, control of temperature, and simulation of ischemia and reperfusion with measurements of the effects of such interventions on both populations of cells and individual myocytes.

This report describes the experimental system and characterizes contractile responses of cardiac myocytes isolated from adult rat hearts to differences in temperature, changes in stimulus parameters, rapid exposure to caffeine, and simulated ischemia and reperfusion.
shaped cells obtained per heart (average = 5.5 × 10^6). Initial viability of myocytes was determined as the percentage of rod-shaped cells in the total sample of cells that retained the ability to exclude the vital dye trypan blue (average = 82 ± 1%). The cell suspension was then returned to the 37°C water bath for calcium addition. This was achieved in five successive steps over a 30-min period to a final concentration of 1.0 mM CaCl_2. Viability was reassessed at the end of this procedure (average = 61 ± 1%).

After isolation, cardiac myocytes were settled and resuspended at room temperature two times in a normal Tyrode solution containing (in mM) 140 NaCl, 5.0 KCl, 1.0 MgCl_2, 5.0 HEPES, 2.0 CaCl_2, and 10.0 glucose, and pH was adjusted to 7.4. The solution was pregassed with 100% oxygen. All preparations were stored at room temperature for at least 30 min before being loaded into the perfusion chambers. All measurements were taken within 6 h of myocyte isolation.

Multitrack suffusion system for studies of myocyte function. The new system for studying mechanical properties of isolated cardiac myocytes used in this study is shown in Fig. 1. Myocytes suspended in normal Tyrode solution were drawn into microhematocrit capillary tubes, which served as “test chambers” for evaluating contractile behavior. The ends of each capillary tube were attached via Silastic tubing between two four-channel tubing manifolds. Specific bathing solutions were gassed in elevated reservoirs and could be selected for perfusion through the four parallel test chambers either by manually adjusting stopcocks or electronically by activating solenoid switches in the inflow tubing upstream of the test chambers. The outflow of the downstream tubing manifold passed through a 22-gauge needle that provided a resistance sufficient to maintain overall flow rate at ~6 ml/min. The capillary tubes were viewed on the movable stage of an inverted video microscope on either low (~10) or high power (~40) to visualize either populations of cells or single cells adhered to the bottom of the tubes.

When the temperature was to be regulated above room temperature (37°C or 27°C), the system was enclosed in a heatable, thermostated Plexiglas and styrofoam case, which fit over the reservoirs, the connecting tubing, and the myocyte-containing capillary tubes above the microscope stage. The temperature was then adjusted to the chosen level.

The heated enclosure was actually a circular ductwork system that was mounted on the microscope stage and moved with it. Air flowed in a closed circuit formed by 1) a horizontal duct across the top of the microscope stage, 2) a vertical duct leading upward past one side of the microscope lamp, 3) a horizontal duct passing above the lamp, and 4) a vertical duct leading downward on the other side of the lamp. A fan (Comair-Rotron standard electronic chassis model FN12B3) located in the upward vertical duct produced continuous and rapid air circulation around the circuit. Heat was supplied by a 3” x 5” self-adhesive electric heating pad (Minco Products no. 5665) and actively controlled by a temperature controller (Minco Products CT 15). The temperature in the outflow manifold of the test chamber apparatus was monitored with a small thermocouple (BAT-12, Bailey Instruments) and recorded as the experimental temperature in the test chambers.

The arrangement allowed free external access to the microscope and stage controls. Moreover, the rapid internal air circulation scheme greatly assisted in solving the difficult problem of maintaining a single constant temperature throughout any heated enclosure. Myocytes were allowed to settle in the capillary tubes for about 5 min before perfusion of the tubes was begun. During this time some of the myocytes attached quite firmly to the glass and remained attached when perfusion with oxygenated normal Tyrode solution was initiated. After 30 min of perfusion, each capillary tube contained between 50 and 150 viable contracting, rod-shaped myocytes. Care was taken throughout all steps in each protocol to prevent gas bubbles from passing through the tubes because these were immediately toxic to the cells (as made evident by rapid implosion, rounding up and sometimes detachment from the glass). Bubbles formed in the inflow tubing were trapped and intermittently “bled out” through a bubble-trap sidearm of the fluid line upstream to the test chambers.

The effects of several experimental interventions are included in this report to characterize the behavior of normal rat isolated cardiac myocytes in this system.

Population responses to electrical field stimulation. Electrical field stimulation of the cells was achieved by applying voltage from a Grass S44 stimulator to stimulating electrodes attached to stainless steel connectors in the inflow and outflow lines at each end of the capillary tubes. Stimulus
parameters in most of these experiments were set at 70 V and 7 ms. Measurement of the population response to a given set of stimulus parameters was achieved by monitoring the percentage of rod-shaped myocytes that contracted in response to the stimulus. The effect of changes in voltage intensity on the percentage of cells responding to the stimulus is reported in this study.

Mechanical responses of single myocytes to field stimulation. Vigorously contracting, rod-shaped myocytes (arbitrarily limited to those with lengths greater than 3 times their diameters) were chosen for studies of single-cell mechanical shortening capabilities. Myocyte lengths were determined by standard video edge-detection methods (Crescent Electronics) and captured on-line using PowerLab/410 (AD Instruments). Although only one cell could be monitored at a time, it took only a few seconds to switch the view between cells in a given chamber and only 1–2 min to switch the view between chambers. Thus it was possible to measure mechanical activity of separate experimental groups of cells within a very short time period. In the initial experiments reported here, myocytes were stimulated at 1.0 Hz, and steady-state twitch characteristics were determined after 30-min equilibration at 37°C, 27°C, or 22°C.

Caffeine contractures. Caffeine contractures have been used to estimate characteristics of some calcium-dependent processes involved in cardiac muscle contraction and relaxation (1, 2, 9, 15, 16, 21, 22). In the experiments reported here, such contractures were induced by adapting the method of McCall et al. (13) to our perfusion system. (In these studies, cells were loaded into only one of the test chambers, and the other 3 were plugged.) Once a cell was selected for study, responses to 1.0-Hz field stimuli were recorded, and then the stimulus was abruptly stopped. Within 2–5 s, the perfusate solution was abruptly switched to one containing 10 mM caffeine. (Given that the length and diameter of a capillary tube is 75 mm and 1.2 mm respectively, a flow rate of 6 ml/min is estimated to change the entire chamber volume within 850 ms and that the passage of the wavefront of caffeine-containing solution over a given myocyte occurs within ~1.6 ms). Application of caffeine in this fashion evokes an immediate release of calcium from sarcoplasmic reticular stores and prevents its reuptake (2, 13, 22). Thus the magnitude of the contracture has been taken to reflect the amount of calcium stored in the sarcoplasmic reticulum, and the time course of subsequent relaxation has been taken to reflect the combined effect of all nonsarcoplasmic reticular calcium removal processes in the cell (i.e., the Na+/Ca2+ exchanger, the mitochondrial uniporter, and the plasma membrane Ca-ATPase).

In some of the experiments after the first caffeine contracture of a given cell is measured, the chamber was perfused again with normal Tyrode solution and the cell stimulated as before for 2 min (during which time the contractile response returned to its previous steady-state characteristics). The bathing solution was then switched to one containing no sodium and no calcium, and the field stimulation was stopped for 2 min. This “modified” solution contained (in mM) 140 LiCl, 5.0 KCl, 1.0 MgCl2, 5.0 HEPES, 1.0 EGTA, and 10.0 glucose, and pH was adjusted to 7.4. At the end of this period, a second caffeine contracture was evoked by abruptly switching to caffeine-containing (10 mM) “modified” solution. The absence of sodium prevents normal operation of the Na+/Ca2+ exchanger and eliminates that route of calcium removal from the cell following caffeine-induced contracture (2, 13). Thus the magnitude of the second caffeine contracture has been taken to reflect the amount of calcium stored in the sarcoplasmic reticulum, and the time course of the subsequent relaxation has been taken to reflect the effects of the remaining calcium removal processes in the cell (i.e., the mitochondrial uniporter and the plasma membrane Ca-ATPase).

After these caffeine contractures of a given cell were recorded, the solution was switched back to normal Tyrode, and after a brief reequilibration period (2–5 min), the procedure was repeated on other cells. Experiments reported here were conducted at 22°C.

Myocyte responses under conditions simulating ischemia and reperfusion. This protocol was adapted from one described by Maddiford et al. (12) in which isolated cardiac myocytes were exposed to low-flow, ischemia-mimetic conditions for various intervals with subsequent reperfusion. Our closed-system capillary tube arrangement allowed us to impose sustained periods of hypoxia and mimic true ischemia by totally stopping the flow while we continued electrical field stimulation of the cells. For these studies, capillary tubes containing isolated cardiac myocytes were initially perfused with well-oxygenated normal Tyrode solution, and myocytes were continually stimulated at 1.0 Hz at either 22°C, 27°C, or 37°C. At the end of a 15-min equilibration period, the capillary tubes were observed at low power (>10) for determination of the percentage of rod-shaped cells that responded to the stimulus. Specific cells in each capillary tube were identified and visualized at high power (>40) for analysis of contractile activity using video edge-detection methods.

After the steady-state responses of the myocytes under these control conditions were recorded, the solution perfusing the capillary tubes was switched to one that simulated ischemic conditions. This solution contained all the components of the normal Tyrode solution except it had no glucose and no insulin, pH was 7.0, and argon was bubbled through the solution (for 45 min) to reduce the oxygen content to negligible levels. Tubes were perfused with this ischémio-mimetic solution for 2 min, and then the flow through the tubes was stopped for a prolonged period of time (90 min at 22°C and 27°C and 45 min at 37°C). Stimulation of myocytes at 1.0 Hz continued throughout the solution changes and the entire ischemic period. This protocol mimics an in vivo ischemic situation in that with continual stimulation and cessation of flow, the microenvironment surrounding individual cells will progressively deteriorate. Myocytes were periodically monitored during the ischemic period for overall viability, responsiveness to the stimulus, changes in rest lengths, and twitch characteristics.

At the end of the ischemic period, flow of normal, well-oxygenated solution was re instituted, and myocytes continued to be stimulated for another 30 min. All variables were measured again at the end of this reperfusion period.

Data analysis. Data are reported throughout as means ± SE. Statistical comparisons of data obtained under different testing conditions was achieved by applying two-tailed Student’s unpaired or paired t-tests. Significant differences were declared at P < 0.05.

RESULTS

Steady-state twitch characteristics of individual myocytes at 37°C, 27°C, and 22°C. Characteristics of the steady-state twitch response to 1.0-Hz electrical field stimulation at the end of an initial 30-min equilibration period at three different temperatures are indicated in Table 1. The average of responses from six to nine myocytes was determined for each separate preparation of myocytes. The average initial resting
length of myocytes studied was 126 ± 2 μm with no significant differences between groups. Not unexpectedly, as the temperature decreased, the extent of myocyte shortening in response to the field stimuli increased, the time course of shortening was prolonged, and the maximum rates of shortening and relaxation slowed.

Population responses to field stimulation. The intensity of the field stimulation directly influenced the percentage of rod-shaped myocytes that responded. These studies were conducted at 22°C with stimulus duration at 7 ms and included seven experiments with 137 ± 22 total myocytes monitored per experiment. Not surprisingly, decreasing the stimulus voltage from 70 to 60, 50, and 40 V resulted in a decrease in the percentage of rods responding from 72 ± 3% to 60 ± 4%, 50 ± 4%, and 28 ± 5%, respectively. Increases in voltage above 70 V had little effect on the percentage of cells responding but tended to promote electrolysis at the points of electrode attachment and bubble formation. Large bubbles could either occlude the fluid pathway between electrodes and block transmission of the field stimulus or could break loose and pass through the tube, causing myocyte death.

In experiments done at 37°C and 27°C, the percentage of myocytes responding to stimulus parameters of 70 V and 7 ms was 77 ± 3% and 75 ± 2%, respectively. In another set of experiments assessing the long-term stability of the preparations under steady-state conditions, the percentage response to constant stimulus of 70 V and 7 ms at 1.0 Hz did not change significantly over 2 h (from 72 ± 8% to 70 ± 3%). Thus the percentage of myocytes responding to a given field stimulation was not significantly influenced by either temperature or perfusion time.

Caffeine contractures of individual myocytes. Records of caffeine contractures obtained from a single myocyte in the presence and absence of sodium in the bathing media are shown in Fig. 2 in comparison with the normal twitch response of that myocyte. Characteristics of these contractions are summarized in Table 2. Note that the caffeine contractures are larger and longer than the twitch responses and that the absence of sodium and calcium in the bathing solution significantly increases the contractile amplitude and prolongs the contraction and relaxation times.

Ischemia-reperfusion simulations. Responses of isolated cardiac myocytes to simulated ischemia and reperfusion were characterized at 37°C, 27°C, and 22°C. In preliminary experiments conducted at 37°C, it was apparent that the myocytes rapidly deteriorated when exposed to more than 45 min of simulated ischemia with the number of rod-shaped cells decreasing to very low numbers during ischemia and the remainder of the myocytes washing away upon reperfusion. Thus the duration of simulated ischemia was limited to 45 min in experiments conducted at 37°C. When experiments were conducted at 27°C and 22°C, the myocytes could easily withstand 90 min of simulated ischemia. Data in Table 3 show that, at all three temperatures, the total number of rod-shaped cells decreased during ischemia (because of cell death) and during reperfusion (because of washout). The percentage of rod-shaped cells continually responding to the stimulus (70 V, 7 ms, and 1.0 Hz) decreased during ischemia and recovered during reperfusion at 27°C and 22°C. This recovery was not complete, however, when experiments were conducted at 37°C.

Characteristics of the contractile responses of individual isolated cardiac myocytes during simulated ischemia and reperfusion are shown in Table 4. As can be seen by comparing the responses recorded before, during, and after the simulated ischemia at any one of the three temperatures, the amplitude of myocyte shortening decreased during ischemia as did the rates of contraction and relaxation. Furthermore, on reperfusion, the rates of contraction and relaxation decreased even further. Some recovery of contractile amplitude was apparent in experiments conducted at 22°C, but none was apparent at 27°C, and there was actually a further decline in contractile amplitude in experiments conducted at 37°C. Under the conditions used, there were no observed changes in myocyte resting length during ischemia or reperfusion.

To determine whether the decrease in percentage of myocytes responding during ischemia was a result of altered sensitivity to the stimulus, experiments were performed...
conducted in which responses to altered stimulus intensity were investigated at specific time points during ischemia and reperfusion at 22°C. In these experiments, the steady-state stimulus intensity was set at 50 V and, at the intervals shown in Fig. 3, was briefly altered to 40, 60, and 70 V to assess the percentage of myocyte response at each intensity. The results shown in Fig. 3 indicate that at each stimulus intensity, simulated ischemia resulted in a late suppression in the percentage of cells responding to a given stimulus intensity and then a restoration of percentage of cells responding on reperfusion. There was no stimulus-dependent change in the pattern of myocyte response to ischemia or reperfusion. These results suggest that the ischemia-induced decrease in the percentage of myocytes responding might be due to an ischemia-induced increase in myocyte electrical threshold. For example, the suppression in myocyte response to 50-V stimulus observed at 90 min of ischemia could be completely negated by increasing the voltage intensity to 70 V.

**DISCUSSION**

This method of suffusing isolated cardiac myocytes offers several advantages over other techniques used to evaluate cardiac myocyte function. First, the parallel arrangement of four separate test chambers allows for assessment of function of cardiac myocytes isolated from hearts of up to four separate experimental groups during a single experimental protocol and permits immediate comparisons of myocyte responses under identical testing conditions. Second, it is possible to tightly control the extracellular environment of the myocytes by selecting the suffusion solution, its flow rate, the experimental temperature, and the external stimulus conditions. Third, the suffusion solution can be rapidly changed so that all cells in the test chambers are exposed to altered conditions within 1 s. The transit time of the wavefront generated by a solution change over a given cell is estimated to be <2 ms. Fourth, responses of entire populations of cells as well as of individual cells can be monitored and compared by switching from low- to high-power microscope objectives. Fifth, with the exception of the inverted microscope, a stimulator, and the video edge-detection system.

**Table 2. Caffeine contractures compared with steady-state twitches of isolated cardiac myocytes**

<table>
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<tr>
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<th>Steady-State Twitch in Normal Tyrode</th>
<th>Caffeine Contracture</th>
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<tr>
<td></td>
<td>Normal Tyrode</td>
<td>0 Na⁺ and 0 Ca⁺²</td>
</tr>
<tr>
<td>Shortening, % of initial</td>
<td>9.3 ± 0.6</td>
<td>13.6 ± 0.8*</td>
</tr>
<tr>
<td>Peak shortening time, s</td>
<td>0.166 ± 0.004</td>
<td>0.783 ± 0.119*</td>
</tr>
<tr>
<td>Time to 1/2 relaxation, s</td>
<td>2.75 ± 0.35*</td>
<td>11.5 ± 0.8±</td>
</tr>
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</table>

Data are means ± SE. Experimental n = 14 for normal Tyrode solution with 42 cells, n = 6 for solutions with 0 Na⁺ and Ca⁺² with 12 cells. Temperature, 22°C. Caffeine contractures were induced by abrupt change to solution with 10 mM caffeine. *P < 0.05 compared with twitch value (paired t-test). †P < 0.05 compared with value of caffeine contracture in normal Tyrode solution (paired t-test).

**Table 3. Effect of simulated ischemia and reperfusion on percentage of myocytes responding to field stimulation**

<table>
<thead>
<tr>
<th></th>
<th>37°C</th>
<th>27°C</th>
<th>22°C</th>
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<tbody>
<tr>
<td>Total no. rods</td>
<td>% Resp</td>
<td>Total no. rods</td>
<td>% Resp</td>
</tr>
<tr>
<td>Preischemia</td>
<td>Ischemia</td>
<td>Early</td>
<td>71 ± 6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid</td>
<td>70 ± 6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>65 ± 6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reperfusion</td>
<td>53 ± 9*</td>
</tr>
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Data are means ± SE. Measurements during ischemia designated as early, mid, and late at 37°C were made at 15, 30, and 45 min. Measurements at 27°C and 22°C were made at 30, 60, and 90 min. Number of experiments at 37°C = 13, at 27°C = 9, and at 22°C = 8. *P < 0.05 compared with preischemic value (paired t-test). †P < 0.05 compared with value obtained at 37°C (unpaired t-test).

**Table 4. Effect of simulated ischemia and reperfusion on contractile characteristics of isolated cardiac myocytes**

<table>
<thead>
<tr>
<th></th>
<th>37°C</th>
<th>27°C</th>
<th>22°C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>+dL/dt max, μm/ms</td>
<td>-dL/dt max, μm/ms</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Preischemia</td>
<td>Ischemia</td>
<td>7.17 ± 0.45</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>15 min</td>
<td>5.80 ± 0.51*</td>
<td>0.32 ± 0.03</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>30 min</td>
<td>4.82 ± 0.35*</td>
<td>0.28 ± 0.03</td>
<td>0.20 ± 0.02*</td>
</tr>
<tr>
<td>45 min</td>
<td>4.55 ± 0.37*</td>
<td>0.25 ± 0.03*</td>
<td>0.20 ± 0.03*</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>15 min</td>
<td>3.46 ± 0.46†</td>
<td>0.14 ± 0.02†</td>
</tr>
</tbody>
</table>

Data are means ± SE. Number of experiments at 37°C = 13, at 27°C = 9, and at 22°C = 8. Number of cells studied per experiment at each interval is 6–9. *P < 0.05 compared with preischemic value. †P < 0.05 compared with value obtained at end of ischemic period. dL/dt max, maximal rate of length change.

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The data obtained using this system are similar to those reported by others. The amplitude and rates of myocyte shortening and relaxation obtained in this study under control conditions (Table 1) are within the same range as those obtained by others using open chambers with myocytes attached to the bottom or to coverslips placed in the chambers (1, 7, 8, 11, 20, 23). Rapid application of caffeine in this study evoked alterations in magnitude and rates of contraction/relaxation of myocytes (Table 2) that were similar to those obtained by others using a micropipette placed near the myocyte being evaluated (1, 13, 14, 20). Simulation of ischemia in this study suppressed myocyte activity, and reperfusion resulted in incomplete recovery of responsiveness and contractile activity (Tables 3 and 4). Although the magnitude of the responses to simulated ischemia and reperfusion depend greatly on the species, the duration of the ischemia, and the specific testing conditions used, the results of the present studies show similar patterns to those reported by others who used low-flow hypoxia (with solutions that mimicked ischemia) followed by reperfusion with well-oxygenated physiological salt solutions (6, 7, 11, 12).

These studies provide baseline information about the effect of changes in temperature on basic contractile properties of isolated rat cardiac myocytes (Table 1). These data are similar to those reported for rabbit and ferret cardiac myocytes (14), in which under control conditions, the amplitude of shortening increased and the rates decreased as temperature decreased. In addition, the present study indicated that changes in temperature did not influence the ischemia-induced decrease in the percentage of myocytes responding to field stimulation (Table 3) or the proportional alterations in the ischemia-induced decreases in contractile amplitude and rates of shortening and relaxation (Table 4). Recovery of myocyte responsiveness to stimulation and amplitude of myocyte shortening during reperfusion were better at 22°C and 27°C than at 37°C (Tables 3 and 4).

A number of techniques have been devised for rapidly changing the bathing solution of isolated cardiac myocytes (1, 10, 18, 19). These techniques have all involved placing a single- or double-barreled micropipette within a few micrometers from a single myocyte and observing various physiological responses (e.g., myocyte shortening, intracellular ion transients, and membrane potential alterations) to either squirting substances directly onto the cell or to changes between two solutions flowing over the cell. The technique described here is not useful for studies of membrane potential with micropipettes because the perfusion chamber is glass enclosed. However, it is useful for applying rapid changes to a single cell within milliseconds without disturbing flow patterns across the cell as well as achieving rapid changes to an entire population of cells within a second.

Limitations of this system are similar to those of other systems used to monitor cardiac myocyte behavior. First, the firmness of the adherence of the myocyte to the underlying material (glass, in this case) will influence the magnitude of shortening of the cell such that the actual contractile ability is difficult to discern. Second, selection of which particular cells to study among the wide variety of sizes, shapes, and contractile responses that are present in any given experiment is a concern for any experimental protocol using isolated myocytes. In this system, it is possible to mark a position of the test chamber and return to a given cell to compare its responses under a variety of extracellular conditions. With each cell as its own control, variability that might arise from comparing effects of a given intervention using populations of cells can be minimized. Third, the estimation of the time to change the suffusate solution over a given cell ignores the parabolic shape of the interface between the two solutions as the new solution moves through the capillary tube. Thus the actual time for the microenvironment surrounding the cell attached to the capillary wall to equilibrate with the new solution will be slightly longer than predicted. Such situations exist whenever solution changes are made by directing a stream of solution over a cell attached to a wall or bottom of a chamber.

There are some special methodological caution that need to be taken when using this system. First, the cells chosen for a study need to be attached on or near the bottom of the tube. The optical distortion due by the tube curvature can introduce significant measurement errors. (Capillary tubes with square lumens are available and would overcome this difficulty.) Second, shear stresses produced by high flow rates can overcome attachment forces and wash the cells away. Third, the passage of gas bubbles through the test.
chambers can have catastrophic effects on the myocytes. High stimulus intensity promotes electrolysis and bubble formation at the site of electrode attachment. These bubbles can be isolated and “bled off” by placing the upstream electrode on a stainless steel connector in the bubble-trap sidearm of the fluid line. During the ischemic episodes, cessation of flow through the test chambers can be accomplished by blocking the outflow tube, and a low flow of fluid out of the upstream sidearm can wash out any upstream bubbles that formed. Another cause of bubble formation in the line arises from heating the perfusion solutions in the reservoirs. This often occurs in lines from reservoirs of perfusate that are used only intermittently in the experiment. Caution must be taken to “bleed off” the bubbles from these lines via the upstream sidearm before allowing the fluid from these reservoirs to flow through the test chambers.

We have successfully used this technique to assess contractile properties of cardiac myocytes isolated from hearts of rats chronically treated with the antineoplastic agent adriamycin and to compare these properties with those of sham-treated rats (4) The ability to do side-by-side myocyte isolations and contractile evaluations has greatly simplified the conduct of these experiments.

The authors acknowledge the expert technical contributions of Jamie Denninger in isolating the cardiac myocytes and conducting many of the experimental protocols described in the study.

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