Development of a Novel Microperfusion Chamber for Determination of Cell Membrane Transport Properties

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ABSTRACT A novel microperfusion chamber was developed to measure kinetic cell volume changes under various extracellular conditions and to quantitatively determine cell membrane transport properties. This device eliminates modeling ambiguities and limitations inherent in the use of the microdiffusion chamber and the micropipette perfusion technique, both of which have been previously validated and are closely related optical technologies using light microscopy and image analysis. The resultant simplicity should prove to be especially valuable for study of the coupled transport of water and permeating solutes through cell membranes. Using the microperfusion chamber, water and dimethylsulfoxide (DMSO) permeability coefficients of mouse oocytes as well as the water permeability coefficient of golden hamster pancreatic islet cells were determined. In these experiments, the individual cells were held in the chamber and perfused at 22°C with hyperosmotic media, with or without DMSO (1.5 M). The cell volume change was videotaped and quantified by image analysis. Based on the experimental data and irreversible thermodynamics theory for the coupled mass transfer across the cell membrane, the water permeability coefficient of the oocytes was determined to be 0.47 $\mu m \cdot min^{-1} \cdot atm^{-1}$ in the absence of DMSO and 0.65 $\mu m \cdot min^{-1} \cdot atm^{-1}$ in the presence of DMSO. The DMSO permeability coefficient of the oocyte membrane and associated membrane reflection coefficient to DMSO were determined to be 0.23 and 0.85 $\mu m/s$, respectively. These values are consistent with those determined using the micropipette perfusion and microdiffusion chamber techniques. The water permeability coefficient of the golden hamster pancreatic islet cells was determined to be 0.27 $\mu m \cdot min^{-1} \cdot atm^{-1}$, which agrees well with a value previously determined using an electronic sizing (Coulter counter) technique. The use of the microperfusion chamber has the following major advantages: 1) This method allows the extracellular condition(s) to be readily changed by perfusing a single cell or group of cells with a prepared medium (cells can be reperfused with a different medium to study the response of the same cell to different osmotic conditions). 2) The short mixing time of cells and perfusion medium allows for accurate control of the extracellular osmolality and ensures accuracy of the corresponding mathematical formulation (modeling). 3) This technique has wide applicability in studying the cell osmotic response and in determining cell membrane transport properties.

INTRODUCTION

Cryopreservation of biological cells and tissues has been used in many scientific research and medical applications. A major challenge for cryobiology is how to protect cells or tissues from injury caused during addition of cryoprotective agents (CPAs) to the cells before cooling and during freezing and thawing, as well as from injury caused during removal of CPAs from the cells after warming (Mazur, 1990). The addition and removal of cryoprotectant(s) may generate deleterious osmotic volume changes of cells if the procedures for addition and dilution are not carefully designed (Penninckx et al., 1984; Critser et al., 1988; Gao et al., 1995). Cooling and warming rates and flux of water as well as intracellular water content play critical roles in causing cell injury or lethal intracellular ice formation and recrystallization (Mazur, 1990; Toner et al., 1990; Muldrew and McGann, 1990, 1994). Several important cell membrane transport properties need to be determined, based on fundamental cryobiology principles, to prevent cell injury and optimize cryopreservation procedures for a given cell type. These properties include the cell membrane permeability coefficients of water (hydraulic conductivity, L_p) and of cryoprotective agents (ω) (CPAs; e.g., glycerol, dimethylsulfoxide, propylene glycol, etc.), reflection coefficients (σ) of the cell membrane to CPA(s), as well as activation energies of these permeability coefficients (Mazur, 1990; Toner et al., 1990; Muldrew and McGann, 1993; Gao et al., 1995). Measurement of the kinetics of cell volume change under anisosmotic conditions is crucial for determination of these membrane transport properties (Leibo et al., 1980; McGrath et al., 1992a; Gao et al., 1994).

Various devices have been developed to quantify cell membrane transport properties by means of "stop flow" (Macey and Farmer, 1970), light microscopy (McGrath et al., 1992a; Leibo et al., 1980; Gao et al., 1994), nuclear magnetic resonance (Paganelli and Solomon, 1957), electron paramagnetic resonance (Kleinhans et al., 1992), and electronic sizing (McGann et al., 1982; Liu et al., 1995;

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Gilmore et al., 1995). Among these methods, light microscopy is the only one that has been used to determine membrane transport properties of individual cells without requiring a large cell population. This technique can be used to directly observe, trace, and measure the volume change of a single cell or cells in anisosmotic environments. Using light microscopy, a microdiffusion chamber system developed by McGrath et al. (1989) has been proven to be very useful in determining the water permeability coefficient (L_p) of a number of cell types when only water flows across the membrane (McGrath and Sherban, 1989; Hunter et al., 1992a). This method has also yielded activation energies for membrane hydraulic conductivities that match those determined using alternate methods (Hunter et al., 1992b; Leibo, 1980). In more complex cases, it is of interest to determine the membrane permeability coefficients of both water and cryoprotectant for investigation of the coupled flow of both. Unfortunately, because of difficulties in distinguishing between the kinetics of diffusion across the dialysis membrane used in the microdiffusion chamber and mixing times from flow across cell membranes, in some cases the microdiffusion chamber method appears to have limitations associated with the mathematical complexity and the unknown physicochemical parameters required for accurate parameter estimations (McGrath et al., 1992a). McGrath et al. (1992a) showed that water and DMSO permeability coefficients as well as the reflection coefficient of mouse oocytes determined using the microdiffusion chamber agree very well with data determined using other techniques. However, water and propylene glycol permeability coefficients as well as the associated reflection coefficient of mouse oocytes determined using the microdiffusion chamber were not consistent with data determined using other techniques. To solve this problem, Gao et al. (1994) developed a micropipette perfusion technique to determine the membrane transport properties of mouse oocytes. In their experiments, glass micropipettes were used to hold only the zona pellucida of an oocyte that was perfused directly with an anisosmotic solution. Using this technique, water, dimethylsulfoxide (DMSO), and propylene glycol (PG) permeability coefficients of the mouse oocytes were determined (McGrath et al., 1992; Gao et al., 1994). However, although the micropipette perfusion technique overcomes problems associated with the microdiffusion chamber method and simplifies the mathematical formulation, it can only be applied to cell types with an outer "shells" (e.g., zona pellucida for mammalian oocytes) or a nonliving cell wall to which the pipette can be affixed. This largely prohibits or precludes the applicability of this technique to many other cell types lacking outer shells. This limitation served as a major motivation for the development of the present, novel microperfusion chamber.

In this paper, we present the design of the microperfusion chamber and corresponding manipulation procedures. Two different cell types were used in testing and validating the microperfusion chamber method: 1) mouse oocytes, and 2) golden hamster pancreatic islet cells. The membrane transport properties of these cells were determined using the microperfusion chamber. These determined membrane transport property values agreed well with the data obtained using either a micropipette perfusion or microdiffusion chamber approach for oocytes or an electronic sizing instrument, a Coulter counter, for the islet cells.

MATERIALS AND METHODS

Source of mouse oocytes

Female ICR mice (7–8 weeks old) were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and used throughout the oocyte experiments. The mice were superovulated by injection of 5 units pregnant mares' serum gonadotropin (Diosynth Inc., Chicago IL) intraperitoneally followed 48 h later by a second injection of 7.5 units human chorionic gonadotropin (Sigma Chemical Co., St. Louis MO) (Critser et al., 1987). Mouse oocytes were collected 16–17 h after human chorionic gonadotropin injection and used for the experiment within 30 min to 2 h. A modified Tyrode's solution buffered with Hepes (TALP-Hepes) (Bavister et al., 1983), supplemented with 4 mg bovine serum albumin ml^{-1} (pH = 7.4) and kept at 37°C, was used to wash and maintain the oocytes during micromanipulation. The osmolality of the TALP-Hepes solution was 286 mOsm. The cumulus oophorus cells were separated from the oocytes using hyaluronidase (1 mg · ml^{-1}) (Sigma; H-2251). After hyaluronidase treatment, the oocytes were washed using the TALP-Hepes solution before the microperfusion procedures.

Isolation of islets from hamsters

Unless stated otherwise, all chemical reagents were obtained from Sigma (St. Louis, MO). Collagenase P was purchased from Boehringer Mannheim (Indianapolis, IN). Cell culture reagents, including Hanks' balanced salt solution, Medium 199, fetal bovine serum and 0.25% trypsin-EDTA, were purchased from Gibco (Gaithersburg, MD). Hamster pancreatic islets were isolated as previously described by Gotoh et al. (1987). Briefly, 6- to 8-week-old golden hamsters (Harlan Sprague-Dawley, Indianapolis, IN) were anesthetized via inhalation of Metofane (Methoxyflurane; Pitman-Moore, Mundelein, IL). The common bile duct was cannulated under a stereo microscope with a polyethylene catheter (PE-10 tubing; CMS, Houston, TX), through which ~3–4 ml of cold (1–4°C) M-199 medium containing 0.7 mg/ml collagenase P was injected slowly until the whole pancreas was swollen. The pancreas was excised and digested at 37°C for ~50 min in M-199 medium containing 100 μg/ml penicillin G and 100 μg/ml of streptomycin (Gibco) (no additional collagenase). The digest was washed three times in cold M-199 medium (Gibco) and passed sequentially through a sterile 500-μm stainless steel mesh, then a 100-μm mesh (CMS). Islets were purified by centrifugation through a Ficoll density gradient (1.045, 1.075, 1.085, and 1.100) (Sigma) at 800 g for 10 min. The exocrine cells pelleted at the bottom were recovered separately.

Preparation of single islet cell suspensions

Islets collected from the top two interphases of the Ficoll density gradient were washed three times in cold Medium 199 and examined under a stereo microscope. Hand picking was performed to further increase the purity of the islet preparation. To prepare a single islet cell suspension, islets were washed once with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution, and digested at 37°C for 10–15 min in 0.25% trypsin-EDTA (1 mM) solution (Gibco). When adequate digestion was achieved, the action of trypsin was stopped by addition of serum containing Medium 199. The cell suspension was passed sequentially once through an 18-gauge needle and twice through a 22-gauge needle to ensure a single cell suspension, and then washed once in Medium 199 to remove the trypsin. Viability of the cells was estimated by a combination of fluorescent dye staining with carboxy-

fluorescein diacetate and propidium iodide (Molecular Probes, Eugene, OR). The percentage of cells exhibiting carboxyfluorescein diacetate (viable) and propidium iodide (nonviable) fluorescence was measured using a FACStar Plus Analyzer and FACStar plus research software (Becton Dickinson, Rutherford, NJ) under a 4-W Argon laser operated at 488 nm and 200 nW.

Microperfusion chamber and manipulation procedures

To determine cell membrane transport properties, the history of cell volume variation in response to the change of extracellular osmotic conditions needs to be measured (McGrath et al., 1992a; Gao et al., 1994). To measure cell volume changes as a function of time, four technical requirements must be satisfied if light microscopy is used: 1) The change of the extracellular condition must be feasible, rapid, and controllable. 2) The cell must be held at a fixed location under the microscope during the change of the extracellular medium (otherwise, the cell could be either out of focus under the microscope or disappear entirely from the microscopic optical field). 3) The cell response (volume change) to the change of extracellular condition must be measurable and resolvable (in time and space). 4) The temperature of the cell suspension must be controllable. The microperfusion chamber technique used in this study was designed to meet all these requirements.

The design and structure of the microperfusion chamber system is shown in Fig. 1, *a* and *b*, including a microperfusion chamber, a temperature controller, and a Hamilton syringe. In experiments, a cell suspension with one cell or more cells is first loaded at the solution inlet and then aspirated into the chamber cavity (height: 2 mm, diameter: 1 mm, volume: 1.6 μl) using the Hamilton syringe. With continued aspiration, part of the cell suspension solution moves out of the chamber, but the cell or cells remain on the transparent porous membrane at the bottom of the cavity (polycarbonate screen membrane; Poretics Co., Livermore, CA; membrane thickness: 10 μm, pore diameter: 3 μm, pore density: 2×10^6 pores/cm^2). A perfusion medium (anisotonic solution with or without permeating solutes) is then loaded at the solution inlet and made to pass the cell or cells by aspiration. The perfusion flow rate is controlled using the Hamilton syringe and by adjusting the rpm of a DC motor connected with the Hamilton syringe (Fig. 1 *a*). The total volume of the medium used to perfuse the cell(s) can be measured by reading the scales on the Hamilton syringe. In the present study, the rpm was 60. Based on the Hamilton syringe reading, the original isotonic solution in the perfusion chamber cavity (1.6 μl) was quickly replaced by the new perfusion medium within

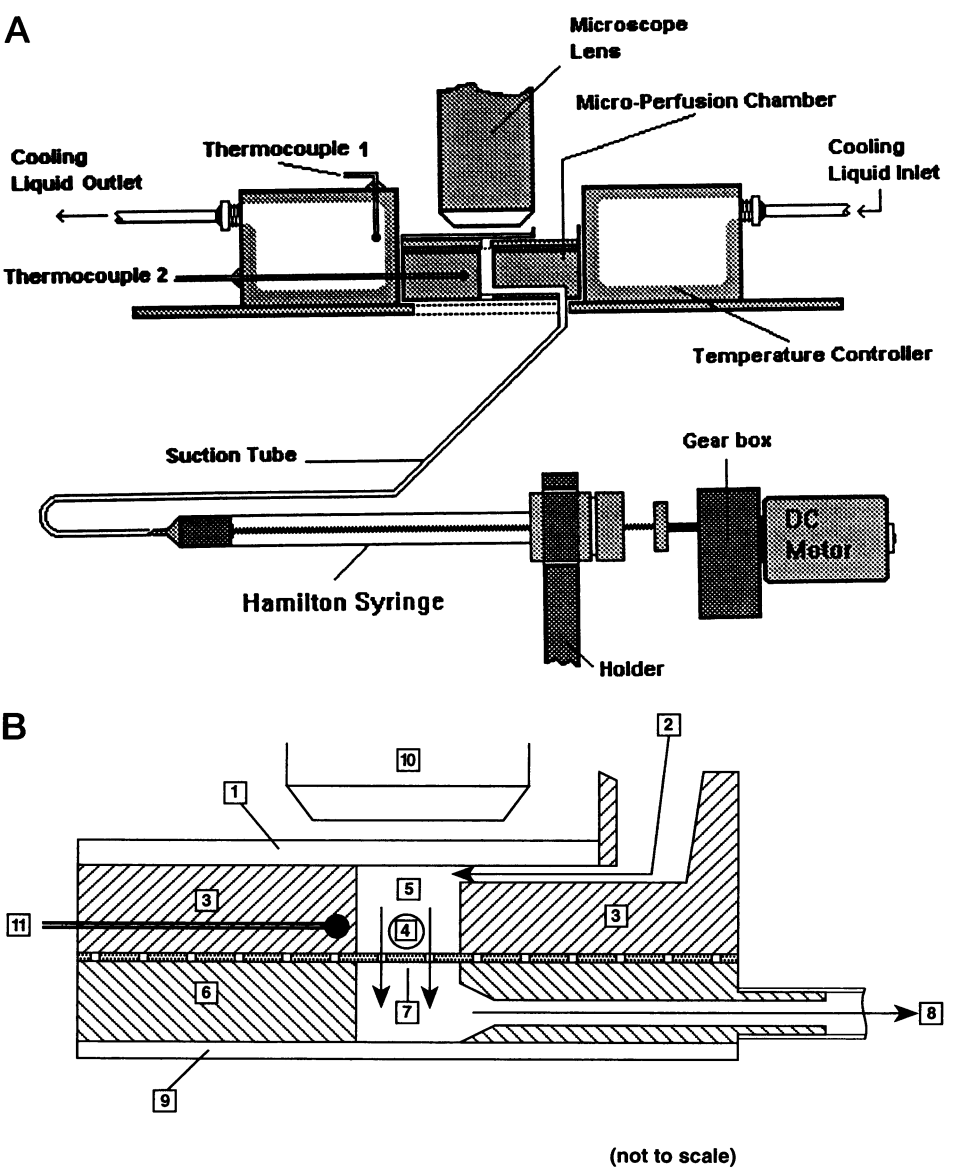

FIGURE 1 Schematic diagram showing (*A*) the setup of the microperfusion chamber system and (*B*) the structure of the microperfusion chamber. 1: glass slide; 2: inlet of the perfusion solution; 3: upper metal body of the chamber; 4: a cell; 5: the chamber cavity/space; 6: lower metal body of the chamber; 7: transparent porous membrane; 8: outlet of the perfusion solution; 9: glass slide; 10: microscopic lens; 11: thermocouple.

0.5 s. The small size of the chamber cavity and downward flow of the perfusion medium immobilizes the cells in the chamber during the perfusion process. The cells are continuously perfused for another 30 s. Over 90 μl of the perfusion medium runs through the cells during the perfusion. Before, during, and after the perfusion process, the history of cell volume changes are recorded by a video camera until osmotic equilibrium is achieved.

Temperature control

The perfusion chamber and cell suspension in the chamber cavity are cooled (or heated) by using a temperature controller (Fig. 1 *a*) to reach an equilibrium temperature (e.g., 22°C in this study). Precooled or heated water is pumped through the jacket of the temperature controller to keep a constant temperature in the microperfusion chamber during the experimental process. The prepared perfusion media are precooled or heated in a temperature-controlled methanol bath (Digital Temperature Controller, Model 9601, Polyscience Co., Niles, IL) to reach the same temperature as that of perfusion chamber. A precooled/heated perfusion media is then loaded in the perfusion chamber and used to perfuse the cells. During the experiment, the temperature variation of the perfusion medium and the temperature change of the microperfusion chamber are monitored using copper-constantan thermocouples (Precision Fine Wire; Omega Engineering, Inc., Stamford, CT). The range of temperature difference between the perfusion medium and the chamber is ± 1°C during the perfusion process. The temperature difference range remains ± 1°C when perfusion is performed at other temperatures, such as 30, 10, or 4°C (Gao et al., unpublished data).

Micropipette perfusion

This technique was previously developed in our laboratory (Gao et al., 1994) to determine membrane transport properties of mammalian oocytes. Micropipettes with an 8- to 10-μm diameter tip opening were made of 1.2-mm glass capillary tubes using a Needle/Pipette Puller (David Kope Instruments, Tujunga, CA) and Microforge MF-1 (Technical Products International Inc., St. Louis, MO). The micropipette was used to apply a negative pressure to hold only the zona pellucida of an oocyte (Fig. 2). (The zone pellucida is a relatively thick, translucent, protein matrix that surrounds the plasma membrane of fully grown mammalian oocytes. It is highly porous and does not serve as a permeability barrier even to very large macromolecules or viruses (Bleil and Wassarman, 1980)). The oocyte was then directly perfused with 1 ml prepared anisosmotic solution with or without CPAs. The temperature of the cell suspension during the perfusion process was controlled by a temperature control device (Temperature controller 1221; Frank E. Fryer Co., Scientific Instruments, Chicago, IL). The dimensional changes of the oocyte before, during, and after perfusion were observed using an inverted Nikon microscope (Nikon, Inc., Garden City, NY) at a magnification of 200X and recorded by a video camera until osmotic equilibrium was achieved.

Experimental design and image analysis

Using the microperfusion chamber or the micropipette perfusion method, three oocytes from each of five mice (15 oocytes in total) were perfused with 900 mOsm NaCl solution or an isotonic (286 mOsm) NaCl solution containing 1.5 M DMSO (adding isotonic NaCl solution to 1.5 M DMSO to make a final 1 liter solution) at 22°C (microscope magnification: 200X). Using only the microperfusion chamber, five pancreatic islet cells obtained from each of six golden hamster (30 cells in total) were perfused with 2X phosphate buffered saline (PBS) (600 mOsm) or 3X PBS (900 mOsm) solutions (solutes in PBS are assumed to be impermeable to the cell membrane) (microscope magnification: 400X). The videotaped images of mouse oocytes and pancreatic islet cells were processed and quantified using a digital image analyzer (The Dynamic Morphology System, Motion Analysis Corp., Santa Rosa, CA) to determine the time dependence of the change in the volume of these cells. Only cells that remained circular in cross-section were analyzed. The average radius of each oocyte was calculated at experimental times of interest by the image analyzer. The average radius was defined as the mean length of the radii from the centroid of the two-dimensional oocyte image to its boundary. Fig. 3 shows an image of a mouse oocyte in 900 mOsm NaCl solution. The cell volumes were computed assuming sphericity. In fact, over 95% of the cells (oocytes or islet cells) maintained near spherical shape before, during, and after perfusion. Less than 5% of cells did not maintain spherical shape and these cells were excluded from image analysis. Data on cell volume history were entered into the parameter estimation portion of the computer software (McGrath et al., 1992a) as a matrix of time and corresponding cell radii.

Thermodynamic modeling and mathematical formulation for coupled and uncoupled mass transfer across cell membrane

The classical formulation of coupled, passive membrane transport was developed by Kedem and Katchalsky using the theory of linear irreversible thermodynamics (Kedem and Katchalsky, 1958). The formulation includes two coupled, first-order, nonlinear ordinary equations that describe the

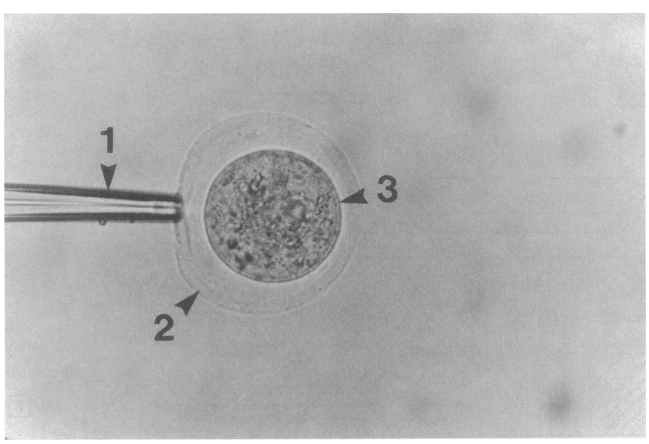

FIGURE 2 Photograph showing a mouse oocyte (at isosmotic condition: 286 mOsm NaCl solution) held with a micropipette. 1: micropipette; 2: zona pellucida of the oocyte; and 3: mouse oocyte. (Reproduced with permission from Gao et al., *J. Reprod. Fertil.* 102:385–392, 1994.)

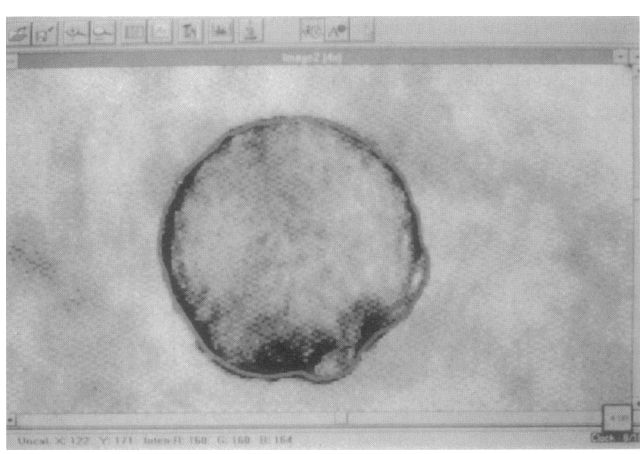

FIGURE 3 Video image of a mouse oocyte (at hyperosmotic condition: 900 mOsm NaCl solution) in the microperfusion chamber.

total transmembrane volume flux and the transmembrane permeable solute flux, respectively. For the case of a solution consisting of a single permeable solute (e.g., DMSO) and other impermeable solutes the equations are:

$$J_v = \frac{1}{A_c}\frac{dV(t)}{dt} = -L_p\{(C^e_{salt} - C^i_{salt}) + \sigma(C^e_{CPA} - C^i_{CPA})\}RT \quad (1)$$

$$J_{CPA} = \frac{1}{A_c}\frac{dN_{CPA}}{dt} = \bar{C}_{CPA}(1 - \sigma)J_v + \omega(C^e_{CPA} - C^i_{CPA})RT \quad (2)$$

where J_v = volume flux, V = cell volume (μm^3), t = time (s), N = osmole number of solute, A_c = cell surface area (μm^2), L_p = water permeability coefficient of the cell membrane ($\mu m/min/atm$), C = concentration of solute (osmolality: Osm/kg(water)), J_{CPA} = CPA flux across the cell membrane, superscript ^{e} = extracellular, superscript ^{i} = intracellular, σ = reflection coefficient of the cell membrane to the CPA, ω = CPA permeability coefficient of cell membrane ($\mu m/s$), T = absolute temperature (K), R = universal gas constant [0.08207 atm * l/(mole * K)], and $\bar{C}_{CPA}$ = average of extracellular and intracellular CPA concentrations (osmolality), $\bar{C}_{CPA} = (C^e_{CPA} - C^i_{CPA})/[\ln(C^e_{CPA}/C^i_{CPA})] \approx (C^e_{CPA} + C^i_{CPA})/2$.

It has been known that mouse oocytes (Leibo, 1980) and hamster pancreatic islet cells (Liu et al., 1995) behave as ideal osmometers (i.e., cell volume is a linear function of the reciprocal of the extracellular osmolality). Therefore, intracellular concentrations of impermeable solute (salt) and permeable solute (CPA) can be calculated (McGrath et al., 1992a) as:

$$C^i_{salt}(t) = C^i_{salt}(0)\left(\frac{V(O) - V_b - \bar{V}_{CPA}N^i_{CPA}(0)}{V(t) - V_b - \bar{V}_{CPA}N^i_{CPA}(t)}\right) \quad (3)$$

$$C^i_{CPA}(t) = \left(\frac{N^i_{CPA}(t)}{V(t) - V_b - \bar{V}_{CPA}N^i_{CPA}(t)}\right) \quad (4)$$

where V_b = osmotically inactive cell volume (μm^3), $\bar{V}_{CPA}$ = partial molar volume of CPA (μm^3), N = number of moles of solute, and 0 = initial condition (t = 0). Initial conditions for $V(0)$, $C^i_{salt}(0)$, $C^i_{CPA}(0)$, $N^i_{CPA}(0)$ are known based on each experimental condition or protocol. In the computer software, it is assumed that: 1) extracellular concentrations of permeating or nonpermeating solutes are constant, and 2) the change of the extracellular conditions caused by the perfusion is instantaneous (i.e., at time = 0^+, extracellular conditions are the same as the perfusion medium). The total volume and surface area of mouse oocytes and pancreatic islet cells at isotonic (286 mOsm) conditions were measured for each cell before perfusion. The shape of oocytes and islet cells is spherical. The average diameter of mouse oocytes used was 76 ± 6.1 μm, and the average diameter of islet cells was 11.4 ± 0.4 μm. The osmotically inactive water volumes (V_b) have been determined to be 20% of isotonic cell volume for mouse oocytes (Leibo et al., 1980) and 40% of isotonic cell volume for pancreatic islet cells (Liu et al., 1995). These values were used in the present study. Using Eqs. 1–4, the kinetics of DMSO/water transport across the cell plasma membrane as well as the cell volume excursion after direct exposure to a solution with a CPA were calculated using a commercial differential equation solver, Diffcham (Version 1.1, Diffcham, East Lansing, MI). The cell volume excursion and water transport through the membrane of cells in an anisosmotic solution without a CPA were calculated using Eqs. 1 and 3 with C_{CPA} = 0.

Parameter estimation methods

The Box-Kanemasu method of parameter estimation was applied (Beck and Arnold, 1977). The method of estimating hydraulic conductivity (L_p), σ, and ω is based on the Gauss method with ordinary least squares (McGrath et al., 1992).

Statistical analysis

Data were analyzed using standard analysis of variance approaches with the General Linear Models procedure of the Statistical Analysis System (Spector et al., 1985). Comparisons were conducted using a protected least significant difference approach (Zar, 1984).

RESULTS

In these experiments, after being perfused at 22 ± 1°C, the mouse oocytes or golden hamster pancreatic islet cells were subjected to a one-step change in extracellular environments. From microscopic observation and image analysis, it was found that: 1) the cell volume changed immediately in response to the extracellular osmotic variation, indicating a fast mix between the perfusion medium and the original cell suspension; and 2) the cells were well focused in the microscope view field during and after perfusion, which ensured high precision in measurement of the cell volume change.

Fig. 4 shows the volume change of a representative mouse oocyte as a function of time after being perfused in the microperfusion chamber using 900 mOsm NaCl. Fig. 5 shows the volume change of a representative mouse oocyte as a function of time after being perfused using an isotonic NaCl solution containing 1.5 M DMSO in the microperfusion chamber. Figs. 4 and 5 also show a comparison of measured and predicted cell volume change histories (best fit between predicted and measured volume changes). The fit between theoretical and experimental data may be or may not be as good as these representative curves in Figs. 4 or 5. The determined water permeability coefficient L_p (mean ± SEM, n = 15) of the mouse oocyte membrane in the absence of DMSO (i.e., uncoupled membrane transport of

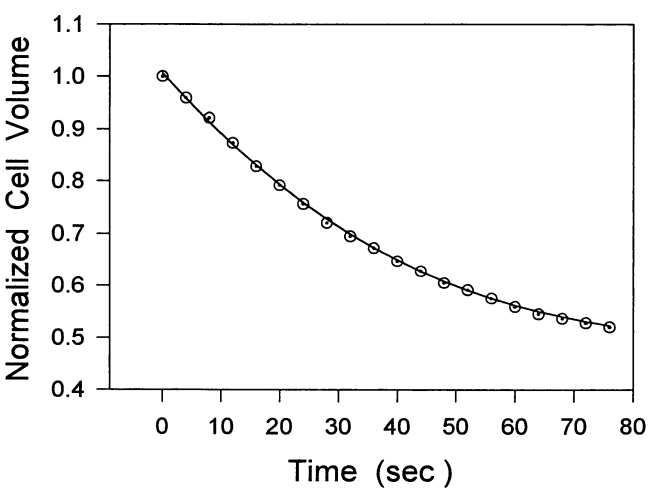

FIGURE 4 Kinetics of cell volume change of a representative mouse oocyte perfused with 900 mOsm NaCl solution at 22°C in the microperfusion chamber and a comparison of measured (⊙) and predicted (——) volume histories. The predictions are based on the best fit L_p value determined here for individual oocytes. The normalized cell volume is defined as the cell volume divided by the original cell volume in the isotonic condition (286 mOsm).

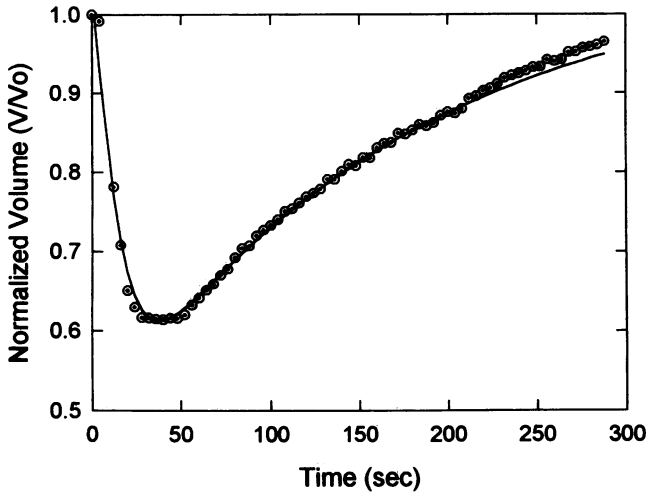

FIGURE 5 Kinetics of cell volume change of a representative mouse oocyte perfused with 1.5 M DMSO solution isotonic with respect to salt (NaCl) at 22°C in the microperfusion chamber and a comparison of measured (⊙) and predicted (——) volume histories. The predictions are based on the best fit L_p, ω and σ determined here for individual oocytes. The normalized cell volume is defined as the cell volume divided by the original cell volume in the isotonic condition (286 mOsm).

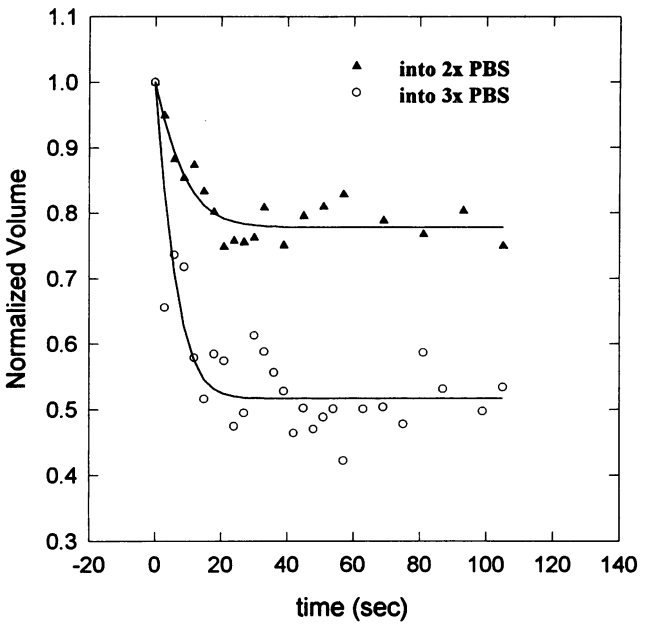

FIGURE 6 Kinetics of cell volume change of two representative golden hamster pancreatic islet cells perfused with 600 (▲) or 900 (○) mOsm PBS solution at 22°C in the microperfusion chamber, and a comparison of measured (▲ and ○) and predicted (——) volume histories. The predictions are based on the best fit L_p determined here for individual islet cells. The normalized cell volume is defined as the cell volume divided by the original cell volume in the isotonic condition (286 mOsm).

water) was 0.47 ± 0.05 $\mu m \cdot min^{-1} \cdot atm^{-1}$ at 22°C. In the presence of 1.5 M DMSO (i.e., coupled water and DMSO transport across cell membrane), the L_p, the permeability coefficient (ω) of DMSO, and the reflection coefficient (σ) have been determined to be 0.65 ± 0.11 (Mean ± SEM, n = 15) $\mu m \cdot min^{-1} \cdot atm^{-1}$, 0.23 ± 0.08 (Mean ± SEM, n = 15) $\mu m \cdot s^{-1}$, and 0.85 ± 0.16 (Mean ± SEM, n = 15), respectively. These data agree well with the oocyte membrane transport properties determined by the micropipette perfusion technique (Table 1).

Fig. 6 shows the volume changes of two representative golden hamster pancreatic islet cells as a function of time after being perfused using 2X PBS (600 mOsm) and 3X PBS (900 mOsm) solutions, respectively. The L_p values for pancreatic islet cells were determined to be 0.27 ± 0.08 $\mu m \cdot min^{-1} \cdot atm^{-1}$ ($\bar{X}$ ± SEM, n = 30).

DISCUSSION AND CONCLUSION

A novel microperfusion chamber system was developed and has been used to determine the membrane transport properties of mouse oocytes and golden hamster pancreatic islet cells.

The determined L_p values for pancreatic islet cells (0.27 ± 0.08 $\mu m \cdot min^{-1} \cdot atm^{-1}$) agree well ($p > 0.05$) with the L_p values, 0.25 ± 0.03 $\mu m \cdot min^{-1} \cdot atm^{-1}$, previously determined using an electronic sizing technique (Coulter counter) (Liu et al., 1995). Table 1 shows a comparison of mouse oocyte membrane transport properties determined by different techniques, i.e., microperfusion chamber (present study), micropipette perfusion technique (present study) and microdiffusion chamber (McGrath et al., 1992a,b), and microscopic method (Leibo, 1980). (In Leibo's experiments, an isotonic mouse oocyte was placed into a hyperosmotic salt solution in a vertical glass capillary tube. Because the density of the isotonic cell was less than the density of the hyperosmotic solution, the cell floated up in the glass tube

TABLE 1 A comparison of mouse oocyte membrane transport properties determined by different techniques

Water permeability coefficients (uncoupled flow)				Water and DMSO permeability coefficients (coupled flow)				
	L_p($\mu m \cdot min^{-1} \cdot atm^{-1}$)	Temperature	Reference		L_p($\mu m \cdot min^{-1} \cdot atm^{-1}$)	ω($\mu m \cdot s^{-1}$)	σ	Reference
Microperfusion chamber	0.47 ± 0.05	22°C	Present study	Microperfusion chamber	0.65 ± 0.11	0.23 ± 0.08	0.85 ± 0.16	Present study
Micropipette perfusion	0.45 ± 0.11	22°C	Present study	Micropipette perfusion	0.79 ± 0.24	0.26 ± 0.11	0.73 ± 0.21	Present study
Microdiffusion chamber	0.48 ± 0.20	20°C	Hunter et al., 1992b	Microdiffusion chamber	0.81 ± 0.23	0.24 ± 0.08	0.80 ± 0.16	McGrath et al., 1992a
Microscopic method	0.44 ± 0.03	20°C	Leibo, 1980	Microdiffusion chamber	0.73 ± 0.20	0.20 ± 0.07	0.88 ± 0.13	McGrath et al., 1992b

Note: Data presented above are mean ± SEM. n = 15 in the present study.
There is no statistically significant difference ($p > 0.05$) among L_p (coupled or uncoupled), ω or σ values determined by different means. However, L_p in coupled flow is significantly different from L_p in uncoupled flow ($p < 0.01$).

during cell dehydration. Cell volume change was observed using a light microscope.) There is no statistically significant difference ($p > 0.05$) among L_p (coupled or uncoupled), ω, or σ values determined by the different methods. However, as shown in Table 1, there is a significant difference ($p < 0.01$) between L_p value in uncoupled flow (i.e., only water permeates across the cell membrane) and that in coupled flow (i.e., water and DMSO both permeate the cell membrane). The L_p in the uncoupled flow is smaller than that in the coupled flow, which is consistent with a previous report (McGrath et al., 1992a) for mouse oocytes. The effect of cryoprotective agents (CPAs) on the L_p has been reported for other cell types, such as human erythrocytes (Toon and Solomon, 1990; Papanek, 1978), lung fibroblasts (Rule et al., 1980), and human spermatozoa (Gilmore et al., 1995). For these cell types, the L_p decreases in the presence of CPAs. The mechanism of the CPA effect on cell membrane water permeability (L_p) is still unknown.

The scatter in the cell-volume-change data, as shown in Fig. 6, reflects the measurement error. Because of the resolution limitation of the light microscopy, the boundary of the cell image is observed as a dark band. It is difficult to estimate the exact boundary of a cell. This measurement error could be relatively large in comparison with cell size if cell dimension is relatively small, such as islet cells (11.4 μm in diameter).

The current study has shown that the microperfusion chamber system is a very useful tool in the study of cell kinetic response to the change of osmotic environment and determination of cell membrane transport properties. The major advantages of the microperfusion chamber technique are: 1) This technique allows the extracellular condition to be readily changed by perfusing a single cell or cells with a prepared medium. To study the response of a given cell to different conditions, the same cell can be easily perfused with different media (different osmolality or different nature of the solutes). 2) The time required for mixing the original cell suspension and perfusion medium is minimized by a quick perfusion process, which allows for accurate control of the extracellular osmolality and simplicity of the subsequent mathematical formulation (modeling). 3) This technique has wide applicability in determination of membrane transport properties of different cell types.

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