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Dynamics of Cell Membrane Permeability Changes at Supraphysiological Temperatures

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ABSTRACT A quantitative fluorescent microscopy system was developed to characterize, in real time, the effects of supraphysiological temperatures between 37° and 70°C on the plasma membrane of mouse 3T3 fibroblasts and isolated rat skeletal muscle cells. Membrane permeability was assessed by monitoring the leakage as a function of time of the fluorescent membrane integrity probe calcein. The kinetics of dye leakage increased with increasing temperature in both the 3T3 fibroblasts and the skeletal muscle cells. Analytical solutions derived from a two-compartment transport model showed that, for both cell types, a time-dependent permeability assumption provided a statistically better fit of the model predictions to the data than a constant permeability assumption. This finding suggests that the plasma membrane integrity is continuously being compromised while cells are subjected to supraphysiological temperatures.

INTRODUCTION

A quantitative understanding of thermal injury is crucial to the production of accurate tissue destruction during clinical hyperthermia as well as devising methods of treatment for thermal and electrical burns. Several reviews are available that discuss the degradative events within the cell caused by a thermal stress (Henriques, 1947; Streffer, 1990; Raaphorst, 1990; Dewey, 1989; Leyko and Bartosz, 1986). Among these events are (1) increased membrane permeability (Cravalho et al., 1992), (2) denaturation of membrane and cytoskeletal proteins (Lepock et al., 1983, 1993), (3) membrane blebbing (Borrelli et al., 1986; Moussa et al., 1977), (4) collapse of cytoskeletal proteins (Welch and Suhan, 1985; Coss et al., 1982; Roti Roti and Laszlo, 1988), and (5) cell lysis (Gershfeld and Muryama, 1988; Moussa et al., 1979; Lloyd et al., 1973). Although many biochemical structures can be damaged at elevated temperatures, the cell membrane is implicated as a key target of the cell injury process and the focus of this work.

A number of studies have been performed to quantify membrane changes in liposomes and cells caused by thermal stress. An experimental study by Kanehisa and Tsong (1978) showed that thermally altered liposome preparations have a maximal permeability at the liquid-crystalline transition of the lipid bilayer. The same finding was demonstrated in a theoretical lipid membrane model by Cruzeiro Hansson et al. (1989). In the case of cells, however, a definitive description of cell membrane changes during hyperthermia has been more difficult to quantify. Moussa et al. (1977) used the kinetics of membrane bleb formation in HeLa cells as an indication of thermal damage. In a more recent study, Borrelli et al. (1986) have shown that the size and distribution of hyperthermia-induced membrane blebbing correlates with cell death at 45.5°C. Cravalho et al. (1992) and Padanilam et al. (1994) used fluorescent dye leakage to determine the kinetics of thermally induced plasma membrane damage. However, a detailed understanding of the real-time changes of the cell membrane at supraphysiological temperatures is still lacking.

The objective in this study was to develop a fluorescence microscopy system to quantify, in real time, the hyperpermeability of the plasma membrane of cultured 3T3 mouse fibroblasts and isolated rat skeletal muscle cells exposed to supraphysiological temperatures. Hyperpermeability of the plasma membrane was determined by monitoring transmembrane fluorescent dye leakage of calcein during exposure to supraphysiological temperatures between 37° and 70°C. Moreover, statistical analysis of our data suggested that the plasma membrane permeability at a given constant supraphysiological temperature was a function of time, suggesting continuous changes within the membrane during hyperthermia.

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MATERIALS AND METHODS

3T3 culture

The mouse 3T3 (National Institutes of Health) fibroblasts used in these studies were obtained from the American Type Culture Collection (Rockville, MD). These cells were cultured in 90% v/v Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% bovine calf serum (Gibco) and 1% penicillin/streptomycin (JRH Biosciences, Lenexa, KS). The cells were kept in a 95% air and 5% CO_2 incubator at 37°C until needed. For experimental preparation, the cells were trypsinized and split in mid-log phase and plated onto autoclaved 12- × 12-mm glass coverslips (Bradford Scientific, Epping, NH) in petri dishes (Falcon, Becton Dickinson, Lincoln Park, NJ). The cells were allowed to attach for 12–16 h before experimentation.

Isolation and culture of rat skeletal muscle cells

The skeletal muscle cells used in this study were isolated from the flexor digitorum brevis muscle located in the hind foot of 3-month-old adult, female Sprague-Dawley rats weighing 150–200 g as described elsewhere (Lee et al., 1992). Cells were harvested by surgical removal of the flexor digitorum brevis, digestion in 0.3% collagenase type II (Worthington Biochemical, Freehold, NJ) for 3 h at 37°C, and subsequent trituration and extensive washing in DMEM (Gibco) supplemented with 10% horse serum (Gibco), 1% penicillin/streptomycin (JRH Biosciences), and 5 μg/ml cytosine-β-D-arabinofuranoside (Sigma Chemical Co., St. Louis, MO), a DNA inhibitor that prevents the proliferation of dedifferentiated fibroblasts in culture. The isolated cells were then diluted in DMEM culture media, plated onto non-tissue-culture petri dishes (Falcon), and stored in a 95% air and 5% CO_2 incubator at 37°C for 2 days before experimentation.

Fluorescent microscopy system

A thermal stage and fluorescent microscopy system were used to conduct thermal injury experiments on mouse 3T3 fibroblasts and isolated rat skeletal muscle cells (Cosman et al., 1989; Padanilam et al., 1994). A schematic of the system is shown in Fig. 1. The thermal stage consists of a convectively cooled microscope stage with a highly conductive sapphire (16 × 16 × 1 mm) piece mounted onto a heating window. This heating window is a glass coverslip (18 × 18 mm) with two thin deposited gold films (18 × 2 mm) on opposite sides that dissipate electrical energy supplied by leads from a thermal controller. The temperature of the cell sample is controlled by a custom designed proportional integral feedback thermal controller (Interface Techniques Co., Cambridge, MA). A thermocouple (Omega Engineering, Stamford, CT) inserted between the heating window and sapphire piece monitors the temperature of the cell sample.

The coverslip containing the 3T3 cells was placed directly on the sapphire piece. The coverslip was surrounded by a thin bead of silicone grease (Dow Chemical Co., Midland, MI), and a drop of medium was placed on the cells and another coverslip was placed on top to seal the sample. As skeletal muscle cells do not attach to the coverslip, one to two isolated skeletal muscle cells were directly placed onto the sapphire piece and covered with a top coverslip. The thickness of the suspending media was approximately 100 μm. In a typical experiment, a given cell was monitored at ambient temperature for approximately 5 min to establish baseline levels of cell intensity, and then the temperature of the cell was raised in approximately 2 s to a temperature between 37° and 70°C. The cell was monitored up to 30 min or until no further fluorescent leakage was observed.

Fluorescent images of cells exposed to supraphysiological temperatures were observed and digitally recorded with a Zeiss Axiovert microscope (Zeiss, Thornwood, NY), equipped with an achromat objective, high pressure mercury (Hg) lamp, computer-controlled shutter (Micro Video Instruments, Avon, MA), low light video camera (Silicon Intensified Transmittance, Hamamatsu Photonics, Hamamatsu City, Japan), and a Sony LVR-5000 optical memory disk recorder. Image-1 software (Universal Imaging Corp., West Chester, PA) was used to control the shutter, image acquisition, and quantitative analysis of the recorded digitized images. For intracellular fluorescent intensity measurements, a region was traced along the cell boundary and the average intensity within the traced region was quantified. The background intensity of the image was also measured in a similar manner for a same size region. This background intensity, which reflects the amount of autofluorescence present, was subtracted from the average intensity of the cell at each time point sampled. The corrected intensities were then normalized with respect to the initial corrected intensity.

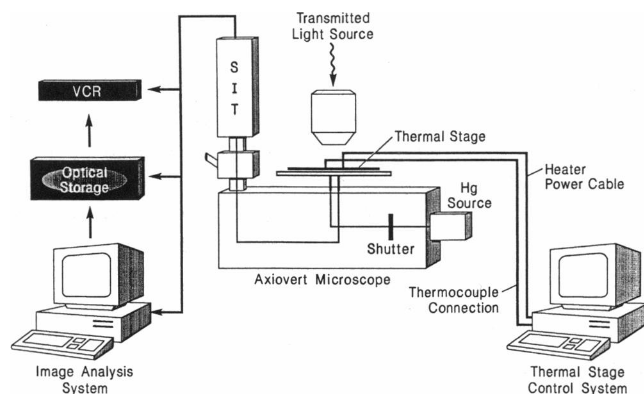

FIGURE 1 Schematic of the components of the fluorescent thermal microscopy system.

Fluorescent dye loading

The leakage of calcein-AM (Molecular Probes, Eugene, OR), a fluorescent membrane integrity probe, from the cells was used to monitor the hyperpermeability state of the plasma membrane. For thermal injury experiments, the cells were loaded with calcein-AM (5 μM) for an hour before experimentation. Calcein-AM is membrane permeable and virtually nonfluorescent until intracellular esterases catalyze the hydrolysis of the AM portion of the molecule resulting in the intensely fluorescent product, calcein, which fluoresces green (485 and 500 nm). The poly-anionic nature of calcein renders it membrane impermeant, and thus it is retained within the cell under normal physiological conditions. Therefore, an increase in calcein flux across the membrane indicates hyperpermeability corresponding to damage of the plasma membrane.

Calcein permeability measurements

The transport of calcein across the plasma membrane was modeled by using a membrane-limited two-compartment model:

$$V\frac{dC_i}{dt} = -PS(C_i - C_o) \tag{1}$$

where V is the volume of the cell, C_i and C_o are the intra- and extracellular concentrations, respectively, P is the cell membrane permeability, and S is the surface area of the cell. To estimate the membrane permeability from Eq. 1, C_i was related to the measured fluorescent intensity by using the following linear relationship:

$$I(t) = \varphi V C_i(t) \tag{2}$$

where I is the fluorescent intensity and φ is a constant of proportionality.

To investigate the effects of supraphysiological temperatures on the membrane permeability, two variations of the above model were proposed. The first assumed that the permeability was solely temperature dependent and the second assumed that the permeability was a function of both temperature and time. Assuming that $C_i \gg C_o$, the integration of Eq. 1 together with Eq. 2 gives the following expression for constant permeability at a given temperature:

$$\frac{I(t)}{I_o} = \exp\left[-\frac{PS}{V}t\right] \tag{3}$$

If, however, the permeability was assumed to vary linearly with time at a given temperature, as follows:

$$P = \alpha t + \beta \tag{4}$$

then the integration of Eq. 1 yields:

$$\frac{I(t)}{I_o} = \exp\left[-\frac{St}{2V}(\alpha t + 2\beta)\right] \tag{5}$$

where α and β are two empirical constants to be determined from measurements.

Characterization studies

In the absence of cells, various concentrations of the dye were hydrolyzed in a 1:1 potassium hydroxide and methanol mixture. To ensure reproducible

volumes of the hydrolyzed dye, a lithographically produced hemocytometric slide (10 ± 1 μm thick), which holds 2 μl of solution was loaded and observed under the microscope. In the presence of cells, both cell types were continuously exposed to the Hg light source at ambient temperature and the photobleaching of calcein was measured from the loss of fluorescence intensity. In addition, the natural dye leakage was assessed from the loss of fluorescence intensity as a function of time. To insure against photobleaching during natural dye leakage experiments, the fluorescent field was sampled at $\geq$5 min-intervals for each cell type for a 25-min period. For spatial variation across the digitized field, the fluorescence was investigated by measuring the intensity across the microscopic field and across the length of calcein-loaded 3T3 and rat skeletal muscle cells. We also determined whether leakage varied spatially along the plasma membrane by measuring the intensity of fluorescence at the middle and two ends of a skeletal muscle cell at 42° and 50°C. Furthermore, the volume of trypsinized 3T3 cells were measured as a function of time at 45° and 50°C from the projected cell surface area assuming perfect spherical geometry to rule out possible artifacts caused by volumetric changes during calcein leakage experiments.

Data analysis and statistics

Three separate test samples of four to six cells each were used for each temperature with the 3T3 cells, and three test samples from three separate isolations of one to two cells were used for each temperature with the skeletal muscle cells. The error bars in the figures represent the standard error of the mean. Curve fits on the dye leakage curves for each cell type were performed by using Eqs. 3 and 5. The *F*-test was used to statistically determine whether a time-varying or higher order permeability fit is justified on the basis of the following equation (Bevington and Robinson, 1992):

$$F_x = (N - m - 1)\frac{\chi^2_{\mathrm{const}} - \chi^2_{\mathrm{time}}}{\chi^2_{\mathrm{const}}} \qquad (6)$$

where χ^2_{const} is the χ^2 value for the fit of Eq. 3 to the dye leakage curves of the cells at a particular temperature, χ^2_{time} is the χ^2 value for the fit of Eq. 5 to the same curves, N is the number of data points per experiment, and m is the number of parameters in the constant permeability fit (= 1).

RESULTS

Characterization studies

A critical assumption in the present study was that the fluorescent intensity of the calcein in the cell was in direct proportion to the concentration of dye in the cell (Eq. 2). The relationship of fluorescence intensity to concentration of hydrolyzed calcein-AM in the hemocytometric slides yielded a linear relationship between 5 and 60 μM with $R^2 = 0.985$ (data not shown) indicating that this assumption was reasonable.

We next examined photobleaching of calcein. As the intensity loss was $\leq$10% after 2 min of continuous exposure to a Hg light source for both cell types (data not shown), a shuttering scheme was adopted during the actual experiments so that total exposure of the cells to light was always less than 2 min. In addition to photobleaching, the natural leakage of calcein under ambient conditions was evaluated for the two cell types. For the first approximately 20 min, the drop in intensity of the dye inside the cells was negligible for both cell types (data not shown). At 25 min, the skeletal muscle cells showed a <5% drop in intensity, and the 3T3 cells a <20% intensity drop. Given that the duration of most experiments was less than 20 min, the contribution of natural dye leakage was also considered negligible for this work.

The spatial variation of the field for experiments with cells indicated that the intensity was highest in the center of the field and decayed toward the edge of the field. This decay was <10–15% for a typical 3T3 experiment and <5% for a typical isolated skeletal muscle cell experiment. In addition, differential leakage of calcein along the plasma membrane was found to be 1–2% across 3T3 cells and <5% for skeletal muscle cells as a function of time at 42° and 50°C (data not shown), indicating that there was no variation in calcein leakage along the plasma membrane.

The cell volume measurements at 45° and 50°C with suspended 3T3 cells showed that there was no volume change for the duration of calcein leakage experiments (~25 min), indicating that volumetric changes could not account for the observed drop in calcein intensity. Furthermore, experiments with cultured 3T3 cells did not show any signs of volume changes such as rounding up and detachment from the surface. Another plausible explanation for the observed drop in intensity could be metabolic conversion of calcein to a nonfluorescent form that was activated at elevated temperatures. This possibility was ruled out, however, based on the fact that when a membrane sealant (i.e., Poloxamer 188) was used there was no drop in intensity of intracellular calcein for temperatures as high as 50°C (Padanilam et al., 1994)

Calcein dye leakage and permeability experiments

Experiments were performed to determine the kinetics of dye leakage from cells at elevated temperatures. Micrographs depicting dye leakage from 3T3 cells exposed to 40°, 50°, and 60°C at 0 and 5 min are shown in Fig. 2. Higher temperatures resulted in an increase of calcein dye leakage out of the cells (i.e., decrease in fluorescence intensity). Micrographs from a typical experiment depicting dye leakage at different time points from isolated rat skeletal muscle cells exposed to 55°C are shown in Fig. 3. The kinetics of dye leakage from cells were obtained from similar experiments at different temperatures.

The cellular response, as measured by the drop in fluorescent intensity over time, of cultured 3T3 cells and isolated skeletal muscle cells is shown in Figs. 4 and 5, respectively. Fig. 4 *A* shows normalized intracellular fluorescent intensity as a function of time for 3T3 fibroblasts exposed to temperatures between 37° and 55°C. A clear trend emerges, with exposure to lower temperatures giving greater amounts of calcein remaining within the cells. Cells exposed to 40°C maintained the highest fluorescence intensity over the longest period whereas cells exposed to 55°C showed the largest drop in fluorescence intensity. At elevated temperatures, cellular fluorescence was at first stable and was followed by a rapid decrease in fluorescence intensity. This was particularly clear at 40° and 45°C, with fluorescence remaining stable at 21 and 11 min, respectively. Fig. 4 *B* shows normalized fluorescence intensity as a function of time for 3T3 fibroblasts exposed to temperatures between 55° and 70°C. At temperatures above 55°C an average of 40–50% of the

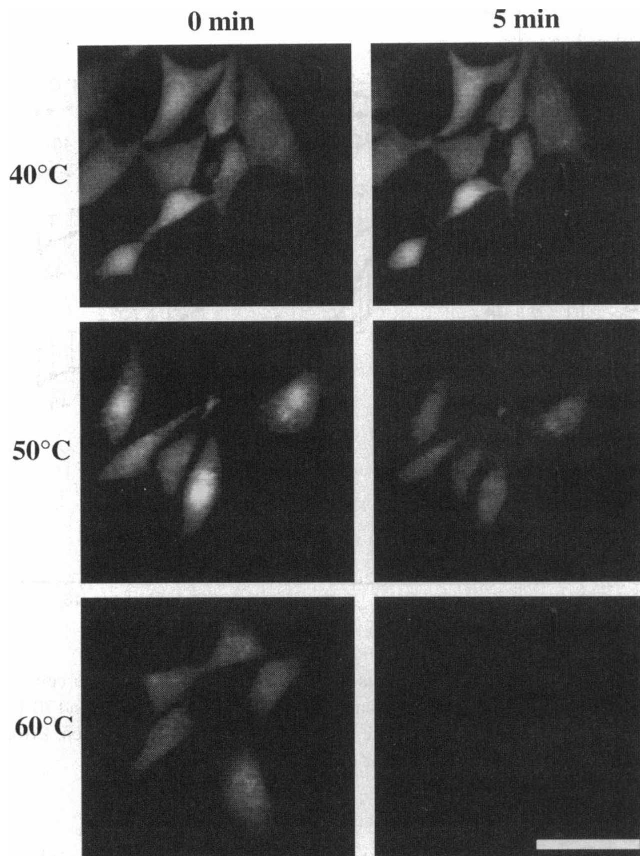

FIGURE 2 Micrographs of representative dye leakage experiments at 40°, 50°, and 60°C for 3T3 fibroblasts at 0 and 5 min. Scale bar represents 50 μm.

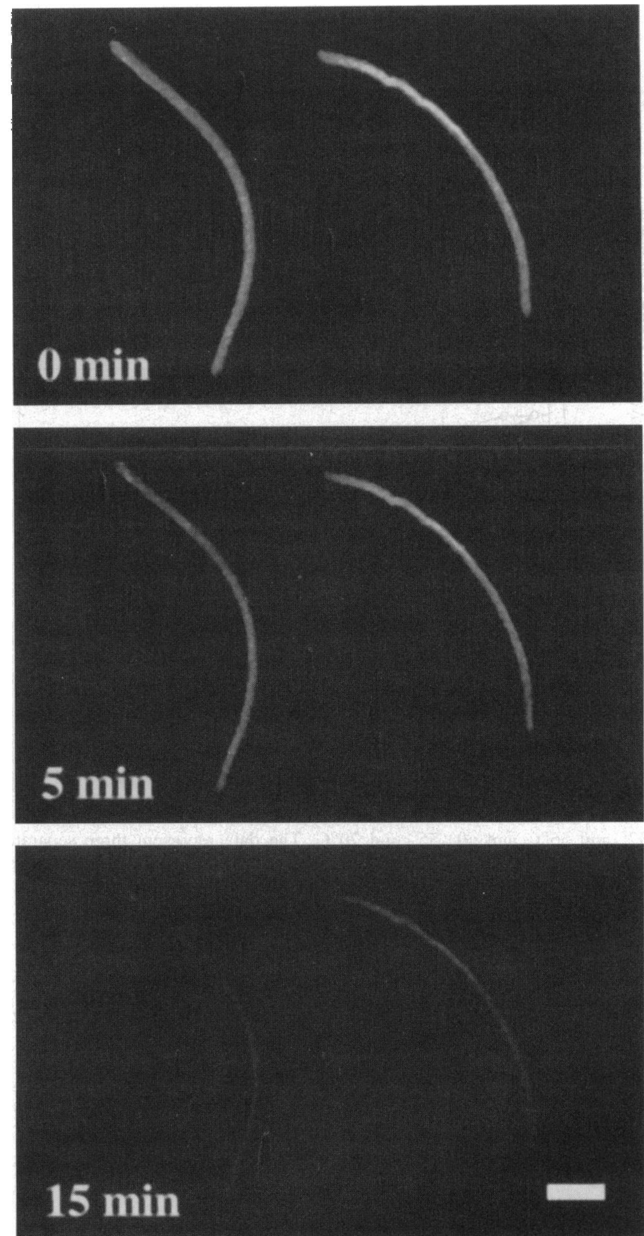

FIGURE 3 Micrographs of representative dye leakage experiments at 55°C for rat skeletal muscle cells after 0, 5, and 15 min. Scale bar represents 100 μm.

calcein dye had leaked out of the cells after 1 min. At ≥55°C, all of the calcein had leaked out of the cells by 2 min. The normalized fluorescent intensity as a function of time for isolated skeletal muscle cells exposed to temperatures from 37° to 70°C is shown in Fig. 5. The drop in cellular fluorescent intensity, and therefore the calcein concentration, shows faster kinetics as the temperature increases. A stable fluorescence intensity similar to that observed in the 3T3 cells can be found in the skeletal muscle cells at temperatures ≤55°C. The duration of the stable period of fluorescence was 2 min at 55°C and 16 min at 40°C. Although some of the error bars overlap at the early time points, at later time points the differences in response to the different temperatures were apparent.

To characterize the effects of elevated temperatures on the integrity of the plasma membrane, two variations of a two-compartment model were used. The first assumed that the permeability of the plasma membrane was constant in time at a given temperature, and the second assumed that the permeability was a function of both temperature and time. When the analytical models in Eqs. 3 and 5 were fit to the calcein leakage curves for the 3T3 and skeletal muscle cells, a statistically significantly better fit to the data was found by using the time-varying permeability in Eq. 5. The curve fits to the data for skeletal muscle cells and 3T3 cells at 50°C are shown in Fig. 6, *A* and *B*, respectively. The *F*-test was used to statistically determine whether the time-varying permeability in Eq. 5 was in fact superior to the constant permeability in Eq. 3 (Bevington 1992). For all supraphysiological temperatures in both cell types, the statistical *P* values were found to be ≤0.001 for temperature ≤55°C and ≤0.05 for 60 and 70°C. These findings showed that the time-varying permeability fit to the data was indeed statistically superior to the constant permeability fit. The values for the empirical parameters for the time-varying permeability given in Eq. 4 are shown in Table 1. The β values showed no clear temperature dependence. However, the second empirically determined parameter of the time-varying permeability fit, α, had a strong

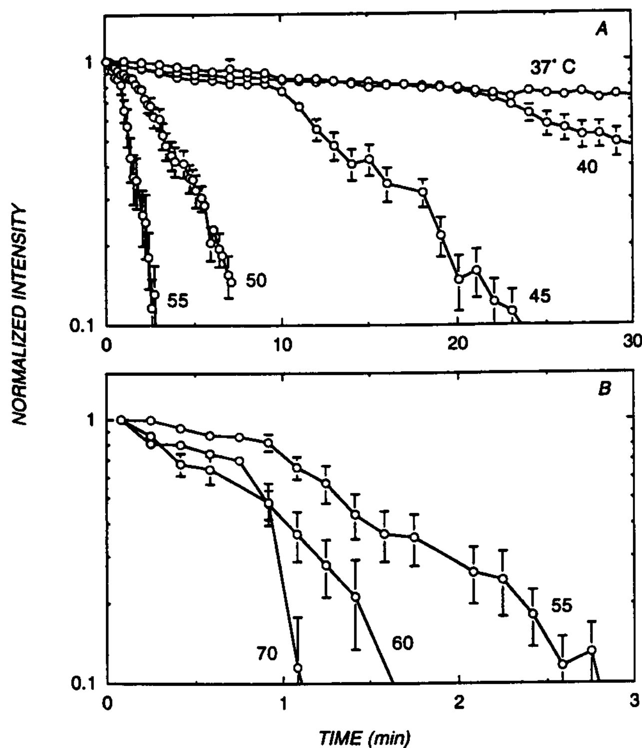

FIGURE 4 Normalized fluorescence intensity of the mouse 3T3 fibroblasts is plotted as a function of time for temperatures ranging between (*A*) 37° and 55°C and (*B*) 55° and 70°C. The data represent three separate preparations of four to six cells each. Error bars represent the standard error of the data.

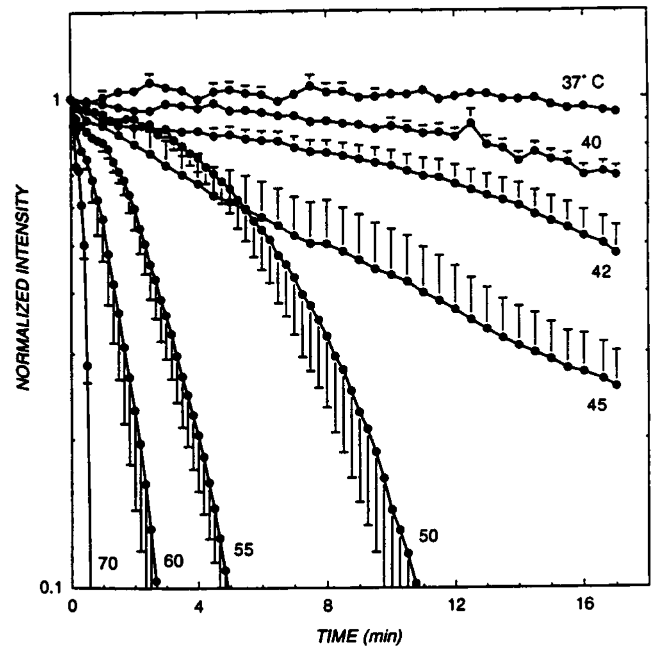

FIGURE 5 Normalized fluorescent intensity of skeletal muscle cells is plotted as a function of time for temperatures ranging between 37° and 70°C. The data represent three separate isolations of one to two cells each. Error bars represent the standard error of the data.

temperature dependence such that higher temperatures led to increased values of α. Data from all experiments reported in Table 1 for both cell types were used to determine the activation energy of the term α. The 3T3 cells and skeletal muscle cells gave activation energies of 70 and 57 kcal/mole, respectively, for the α term in Eq. 4.

DISCUSSION

In this study, plasma membrane hyperpermeability of cultured 3T3 cells and isolated skeletal muscle cells at supraphysiological temperatures was investigated by using a thermal fluorescent microscopy system. The thermally induced dye leakage was recorded by using real-time data acquisition. In general, higher temperatures induced faster kinetics of dye leakage. A simple two-compartment model with either a temperature-dependent, or a time- and temperature-dependent permeability was validated and used to analyze the experimental results. The statistically superior model assumed that the permeability was a function of both time and temperature rather than a function of temperature alone. A time-varying permeability implies that the membrane is being altered continually during the course of thermal insult, becoming more permeable as time progresses.

The lipid component of the plasma membrane has been extensively investigated with regard to its role in increased permeability at elevated temperatures. In experimental as well as theoretical models of lipid bilayers, increased permeability is found to occur particularly around the gel to liquid crystalline phase change (Kanehisa and Tsong, 1978; Mantsch et al., 1988; Cruzeiro Hansson et al., 1989). Although a reversible phase transition in the lipid portion of the plasma membrane of *Mycoplasma laidlawii* was found at 40°C (Melchior et al., 1970), the existence of this phase change is not known for mammalian cells. Kashchiev and Exerowa (1983) investigated thermally driven formation or nucleation of holes in the plasma membrane that may account for increased membrane permeability. Nucleation of holes (or defects) can be driven by voids in the membrane structure or by inclusion of foreign bodies and/or proteins. The two parameters governing the thermodynamics of this defect formation process are believed to be the surface tension of the membrane and the edge free energy of a defect in the phospholipid bilayer (Kashchiev and Exerowa, 1983). If these two parameters are altered in a time-dependent manner during high temperature exposure, then the defect distribution could be altered in a time-dependent manner and lead to a time-dependent permeability that accounts for the calcein leakage curves observed in the present study. Thus, the continuous increase in membrane permeability may be partially caused by time-dependent alterations in the lipid component of the membrane.

The time-varying membrane permeability may also be influenced by alterations in the protein component. Lepock et al. (1983, 1987) have shown that fibroblast membrane proteins denature at temperatures above ~40°C, and the bulk of denaturation occurs between 45° and 65°C during heating at

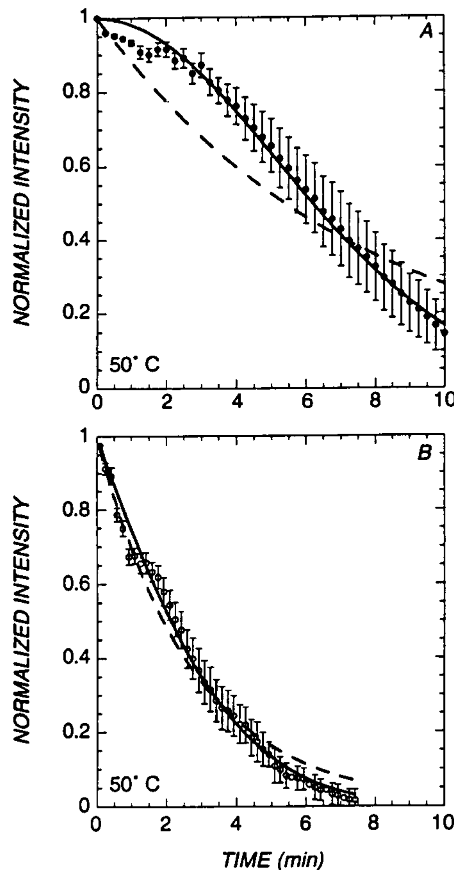

FIGURE 6 Nonlinear curve fits for calcein leakage at 50°C assuming either a constant permeability (dashed line) at the given temperature or a time-dependent permeability (solid line) in (*A*) skeletal muscle cells and (*B*) 3T3 cells.

TABLE 1 Values of the empirical parameters for time-varying permeability given in Eq. 5

T (°C)	3T3 α ($\times 10^9$ m/s^2)	3T3 β ($\times 10^9$ m/s)	Skeletal muscle cells α ($\times 10^9$ m/s^2)	Skeletal muscle cells β ($\times 10^9$ m/s)
40	0.002	0.18	0.003	0.424
45	0.011	0.177	0.004	8.467
50	0.077	7.6	0.049	0.199
55	0.863	8.039	0.167	12.98
60	3.179	38.054	0.349	41.0
70	1.782	27.756	12.21	31.89

a rate of 1°C/min. Thus, it is plausible that the proteins in the two cell types examined in this study are being denatured by the elevated temperatures. Perhaps time-dependent rigidification or denaturation of proteins in the membrane acts as sites for increased liquefaction or nucleation of holes in the surrounding lipid as discussed above, thereby contributing to the observed increase in plasma membrane permeability as time progresses.

In addition to changes in the plasma membrane itself, disruption and collapse of cytoskeletal proteins may be linked to the destabilization and hyperpermeability of the membrane. Welch and Suhan (1985) showed that elevated temperatures resulted in collapse of the vimentin-containing intermediate filament network around the nucleus in rat fibroblasts. Coss et al. (1982) showed that the extent of microtubule disassembly in Chinese hamster ovary cells correlated directly with the thermal dose. Because cytoskeletal alterations at elevated temperature are time dependent, the influence of these effects on the membrane permeability would also be time dependent.

Additional insight into a time-varying membrane permeability at hyperthermic temperatures and hence to the physical meaning of empirical parameters α and β may be gained by discussion of a simple phenomenological model. The overall membrane permeability can be expressed as proportional to an effective porosity (p) such as $P = p\,D/\delta$; where D is the diffusivity of calcein and δ is the characteristic diffusion length for a given cell (Kashchiev and Exerowa, 1983). The only parameter in this expression that can exhibit time dependency to reflect a time-varying permeability is the so-called porosity. We propose a first-order forward rate equation to model the changes in membrane porosity such that $np \xrightarrow{k_1} p$ where np is the physiological nonporous condition to calcein and k_1 is the rate constant of the reaction. One can then characterize the increased porosity ratio as $P = 1 + (p_o - 1)(\exp(-k_1 t))$, where p_o is the initial physiological porosity. When this expression is substituted into the permeability relation as given above, one obtains: $P = D/\delta\,\{1 + (p_o - 1)(\exp(-k_1 t))\}$. By using P_o values at different temperatures (estimated from Table 1 and Eq. 4), it can be shown that the rate constant k_1 is $\ll 1$ between 37° and 70°C for both cell types used in this study, and the overall membrane permeability can then be simplified to a form similar to $P = \alpha t + \beta$, where $\alpha = Dk_1/\delta(1 - p_o)$ and $\beta = Dp_o/\delta$. Thus, the term α reflects the rate at which membrane permeability to calcein changes, and β is the initial permeability of the cell membrane to calcein. This simple analysis should be taken as a qualitative description of a time and temperature permeability, in terms of constants α and β, with some physical meaning as given from the phenomenological porosity model discussed above.

Previous studies on mammalian cells suggest a qualitative trend of increased permeability to ions during hyperthermia. A net loss of intracellular K^+ ions occurs after applying an irreversible heat shock in the range of 41–43°C (Peterson et al., 1979; Yi, 1979; Stevenson et al., 1983; Ruifrok et al., 1985). On the other hand, in sublethal heat shocks, the intracellular K^+ concentration was either recovered or remained unaltered (Yi, 1979; Stevenson et al., 1983; Ruifrok et al., 1985). A number of studies have documented an increase in the active transport of K^+ during hyperthermia presumably to maintain the intracellular K^+ concentration during increased passive loss of the ion at hyperthermic temperatures (Szmigielski and Janiak, 1978; Joiner and Lauf, 1979; Stevenson et al., 1983). More recently, Vidair and Dewey (1986) concluded that heat does not produce any significant changes in the Na^+, K^+, or Mg^{2+} content of the

cells. Their measurements, however, were taken on cells surviving 28 h subsequent to a 45°C 15–35 min heat shock. On the other hand, Yi et al. (1983) took ion measurements at different time points during hyperthermic treatments at 43°C and concluded that the intracellular K^+ and Cl^- content exhibits a decline whereas the Na^+ content does not change significantly. Although these studies indicate that passive ion transport through membranes is most likely enhanced during hyperthermia, dynamic quantitative data on ionic hyperpermeability in single cells is still lacking.

In summary, a quantitative dynamic study on alterations of the plasma membrane permeability in 3T3 fibroblasts and skeletal muscle cells at supraphysiological temperatures has been conducted. The experimental data for dye leakage at supraphysiological temperatures was analyzed with respect to a two-compartment model. This model indicated that the permeability of the plasma membrane varied as a function of time at a given elevated temperature. A number of explanations for the time dependence of the plasma membrane permeability at a given temperature are plausible; however, additional studies are clearly needed to identify the exact mechanism(s) of this time-dependent membrane permeability.

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