Osmometric and Water-Transporting Properties of Guinea Pig Cardiac Myocytes

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Abstract: To elucidate the mechanism of water flux across heart cell membranes, osmotically induced volume changes and sarcolemmal water permeability were evaluated in isolated guinea pig ventricular myocytes by videomicroscopic measurements of cell surface dimensions. Superfusion with anisotonic solution (0.5–4 times normal osmolality) caused a rapid (<3 min to new steady state) and reversible cell swelling or shrinkage mainly because of proportional changes in cell width and thickness. The van't Hoff relationship between relative cell volume and the reciprocal of relative osmolality was linear and predicted an apparent osmotically dead space of ~35% cell volume. The osmotic water permeability coefficient (P_f) measured from the time course of cell swelling/shrinkage was $\sim 22 \mu\text{m} \cdot \text{s}^{-1}$ at 35°C. Arrhenius activation energy (E_a), a measure of the energy barrier to

water flux, was $\sim 3.8 \text{ kcal} \cdot \text{mol}^{-1}$ between 11 and 35°C; this value is equivalent to E_a for free-water diffusion in bulk solution ($\sim 4 \text{ kcal} \cdot \text{mol}^{-1}$). Treatment with 0.1 mM Hg^{2+} , a sulfhydryl-oxidizing reagent, reduced P_f by ~90%, and the sulfhydryl-reducing reagent dithiothreitol (10 mM) antagonized the inhibitory action of Hg^{2+} . E_a measured from Hg^{2+} -treated myocytes ($12.3 \text{ kcal} \cdot \text{mol}^{-1}$) was in the range of that for diffusional water movement through the lipid bilayers ($>10 \text{ kcal} \cdot \text{mol}^{-1}$). Although the observed P_f is small in magnitude, both the low E_a and the sulfhydryl-related modifications of P_f are characteristic of channel-mediated water transport. These data suggest that water channels form a major conduit for water crossing the sarcolemma of guinea-pig heart cells. [Japanese Journal of Physiology, 52, 333–342, 2002]

Key words: cell volume, membrane water permeability, water channel, cardiac myocyte, osmolality.

All animal cells undergo volume expansion or shrinkage because of water flux driven by transmembrane osmotic gradients. The osmotically obliged water moves into and out of the cells primarily by diffusion through the membrane lipid bilayers [1, 2]. Because water movement is constrained by membrane fluidity and lipid organization, the diffusional water transport is small in magnitude ($<50 \mu\text{m} \cdot \text{s}^{-1}$) and characterized by a high ($>10 \text{ kcal} \cdot \text{mol}^{-1}$) Arrhenius activation energy (E_a), a measure of the energy barrier to water flux [1–3]. In specialized tissues, such as renal tubules, eye lens, inner ear, and choroid plexus, membrane water permeability is greatly enhanced by the expression of the channel-forming protein aqua-

porin [3–5]. The expression of water channels is not localized in region where high water permeability is required; it is now established that aquaporin molecules are abundant throughout animal tissues [6, 7]. Recently, diffuse distributions of aquaporin molecules have been identified in several cardiac preparations by immunohistochemical approaches [6–9]. However, the functional significance of their presence in tissues serving a non-water-transporting role is still unclear.

The purpose of this study was to evaluate osmotically induced volume change and sarcolemmal water flux of guinea pig ventricular myocytes. Cell surface dimensions were measured by digital videomicroscopy, and the osmotic water permeability coeffi-

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cient (P_f) was obtained from the time course of cell swelling and shrinkage in response to rapidly imposed osmotic gradients. The results demonstrate that the myocytes exhibit osmometer-like behavior with an apparent osmotically dead space of nearly one-third of their volume. The observed P_f is small ($\sim 22 \mu\text{m} \cdot \text{s}^{-1}$), but E_a is as low as the value for free water diffusion ($\sim 4 \text{kcal} \cdot \text{mol}^{-1}$). These data, together with the pharmacological characteristics of P_f , suggest that channel-mediated water transport plays a key role in the osmosis of guinea pig cardiac myocytes.

MATERIALS AND METHODS

Cell preparation. Experiments were performed in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan. Guinea pigs (250–350 g) were anesthetized with sodium pentobarbital ($50 \text{mg} \cdot \text{kg}^{-1}$, I.P.). Hearts were quickly excised, and single ventricular myocytes were enzymatically isolated as described previously [10]. In brief, the excised hearts were mounted on a Langendorff column and perfused (37°C) with Ca^{2+} -free Tyrode's solution containing collagenase ($\sim 0.1 \text{mg} \cdot \text{ml}^{-1}$; Yakult, Tokyo, Japan). The cells were dispersed and maintained in a modified "KB" storage solution at 4°C . A few drops of cell suspension were placed in a 0.25-ml glass-bottomed chamber mounted on an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan), and superfused ($3\text{--}4 \text{ml} \cdot \text{min}^{-1}$) with bathing solution at 35°C , unless otherwise noted. Only rod-shaped quiescent cells with smooth contours were selected for study.

Solutions. Normal Tyrode's solution contained (mM) NaCl 140, KCl 5.4, CaCl_2 1.8, MgCl_2 1, glucose 10, and HEPES 5 (pH 7.4 with NaOH). Isosmotic (1T) Tyrode's solution was made by replacing 70 mM NaCl with 140 mM sucrose ($319 \text{mosmol} \cdot \text{kg}^{-1}$). Hyperosmotic 1.5T, 2.2T, 2.8T, and 4T solutions were made by adding 140–750 mM sucrose to the 1T solution (485, 689, 900, and $1,235 \text{mosmol} \cdot \text{kg}^{-1}$, respectively); Hyposmotic 0.5T solution was made by omitting sucrose ($166 \text{mosmol} \cdot \text{kg}^{-1}$), and 0.75T solution was made by adding 70 mM sucrose ($240 \text{mosmol} \cdot \text{kg}^{-1}$) to the 0.5T solution. Cl^- -free solution was made by the equimolar substitutions of MgSO_4 for MgCl_2 and of methanesulfonate for other Cl^- . Storage solution contained (mM) KOH 90, KCl 30, KH_2PO_4 30, MgSO_4 3, glutamic acid 70, taurine 20, glucose 20, EGTA 0.5, and HEPES 10 (pH 7.4 with KOH, $310 \text{mosmol} \cdot \text{kg}^{-1}$). The osmolalities of the solutions were verified with

a freezing-point depression osmometer (OM-6050, Arkray, Kyoto, Japan), and the variation was $<10 \text{mosmol} \cdot \text{kg}^{-1}$ for a given type of the solution.

Cell dimensions. Myocyte images were captured on-line with a high-resolution CCD camera (HV-D28S, Nikon) and a Macintosh computer equipped with a video frame grabber at $\sim 0.25 \mu\text{m} \cdot \text{pixel}^{-1}$ resolution and analyzed with image-processing software (NIH Image, National Institutes of Health, Bethesda, MD, USA). Halos around the cell images were minimized by using Hoffman modulation contrast optics (Modulation optics, Greenvale, NY, USA) attached to the microscope, and digital image processing (e.g., filtering and thresholding) was used to optimize the identification of cell profiles when appropriate. The borders of cell images were manually traced with a tablet-type pointing device, and the cell area was measured by counting included pixels. Cell length was measured as the longest axis to obtain the minimum average cell width calculated as area/length. Cell thickness was obtained by focusing at the top and bottom surfaces of myocytes and measured as the distance between the two focal planes by averaging 3–5 measurements for each myocyte. To unambiguously identify the top and bottom surfaces, alumina particles (diameter $1\text{--}2 \mu\text{m}$, Sumitomo Chemical, Tokyo, Japan) placed on the myocyte and the adjacent floor of the chamber were used as a marker.

The reproducibility of the cell width, length, and area measurements was assessed by repeatedly (10 times) tracing and measuring the same images recorded from 10 cells; the deviation of each measurement from the mean averaged $0.94 \pm 0.07\%$ for width, $0.55 \pm 0.04\%$ for length, and $1.29 \pm 0.09\%$ for area. To assess cell thickness measurement, a roving "live" microelectrode filled with 3 M KCl (tip resistance $40\text{--}100 \text{M}\Omega$) and connected to a high-input impedance amplifier was held by the headstage of a hydraulic micromanipulator (WR-3, Narishige, Tokyo, Japan) and moved vertically downward until a small ($<1 \text{mV}$) deflection indicated contact with the cell surface. This measurement was read from the scale on the micromanipulator knob and subtracted from the level read when the microelectrode tip touched the floor of the bath ($1\text{--}3 \text{mV}$ deflection); data were taken as the difference between the floor and 4–6 measurements from different areas of the cell. Cell thickness measurements were made by the focusing method after roving microelectrode measurements; the difference was $7.7 \pm 1.3\%$ of the mean ($n=25$).

Sarcolemmal water permeability. The osmotic permeability coefficient P_f , which measures the ability of water to penetrate biological membranes,

was calculated as described by Solomon [11] and Agre *et al.* [12] from the time course of cell swelling and shrinkage in response to a step change in osmotic gradient across the membrane:

$$P_f = \frac{V_0 \cdot dV_{rel}/dt}{S \cdot V_w \cdot \Delta\pi} \quad (1)$$

where V_0 is the absolute volume under control isosmotic conditions, dV_{rel}/dt is the rate of change in the relative cell volume V_{rel} as a function of time (see Fig. 5), S is the cell surface area, and V_w is the molar volume of water ($18 \text{ cm}^3 \cdot \text{mol}^{-1}$). The osmotic gradient $\Delta\pi$ is calculated as

$$\Delta\pi = \pi_i - \pi_o = \frac{(1 - V_b) \cdot \pi_{i(t=0)}}{(V_{rel} - V_b)} - \pi_o \quad (2)$$

where π is the osmotic pressure with the subscripts o outside and i inside the cell, $\pi_{i(t=0)}$ is the π_i at zero time, and V_b is the osmotically inactive volume fraction (see Fig. 3B). By assuming that $\pi_i = \pi_o$ in the steady state, π_i is taken as equal to π_o at zero time, and π_o refers to the osmotic pressure of the test solution. To estimate absolute cell volume and surface area, myocytes were assumed to be brick-shaped with a cell thickness/width ratio of 0.65 (see RESULTS).

The Arrhenius activation energy E_a , defined by the relation $\ln P_f = E_a/RT + A$, where R is the gas constant, T is absolute temperature, and A is an entropic term, was determined from the slope of a semilogarithmic plot of P_f vs. $1/\text{temperature}$ (Figs. 5B and 6Bb).

Statistics. The results are expressed as means $\pm$ SEM, with n indicating the number of experiments, and comparisons were made by using Student's t -test. An analysis of variance with Bonferroni correction was used for multiple comparisons. The differences were considered significant when $p < 0.05$.

RESULTS

Osmotic properties of guinea pig heart cells

To verify analytical methods for P_f determination and to obtain constants included in Eqs. 1 and 2 (see METHODS), we performed a series of control experiments. The results described in the following subsections also help to characterize the osmotic properties of guinea pig ventricular myocytes.

Cell dimensions under isosmotic conditions. To examine whether myocyte dimensions are stable under control 1T conditions, the cell length, width, and thickness were repeatedly measured during continuous superfusion with 1T solution for 180 min.

Fig. 1. Myocyte dimensions and volume during continuous superfusion with isosmotic 1T solution. Myocytes stored in KB solution were placed in a perfusion chamber and superfused with KB solution for ~ 10 min. Superfusion with 1T Tyrode's solution began at 0 min, and cell dimensions are expressed as relative values to the measurements at 10 min; cell volume was calculated as length $\times$ width $\times$ thickness. $n = 20$ –40 myocytes.

After a 60-min superfusion, cell length was $120 \pm 2 \mu\text{m}$, width $23 \pm 0.5 \mu\text{m}$, thickness $15 \pm 0.5 \mu\text{m}$, and the thickness/width ratio 0.65 ± 0.02 ($n = 100$). Cell volume calculated as length $\times$ width $\times$ thickness (lwt) was $43 \pm 3 \text{ pl}$ ($n = 100$). During 1T superfusion, there were small time-dependent reductions in cell width and thickness, but no significant changes in cell length (Fig. 1). When normalized to the measurements at 10 min, cell width declined to 0.97 ± 0.01 , thickness to 0.96 ± 0.01 , and lwt volume to 0.94 ± 0.02 after 180 min ($n = 40$). The decline in cell thickness closely paralleled the decline in cell width, with the thickness/width ratio being nearly constant at ~ 0.65 (Fig. 1, bottom). However, most changes in cell width and thickness occurred during the first 30 min, and therefore cell volume at 180 min was not significantly different from its 30-min value ($-1.6 \pm 1.0\%$ change, $n = 20$). This suggests that osmotic equilibrium across plasma membranes had been reached during 1T superfusion. To minimize the influence of the time-dependent changes, all data reported below were obtained after ~ 60 min equilibration period in 1T solution. Note that the initial shrinkage was primarily due to a reduction in the cross-sectional area and therefore was unlikely to be due to moderate contracture because both myocyte twitch and contracture are characterized by shortening and widening [13, 14].

Osmotic cell swelling and shrinkage. Figure 2A shows that superfusion with anisotonic solu-

Fig. 2. Myocyte swelling and shrinkage under anisotonic conditions. A: Time courses of the changes in cell dimensions before, during, and after superfusion with hyposmotic 0.5T (Aa) or hyperosmotic 1.5T (Ab) solution. Relative cell volume was calculated as $\text{length} \times (\text{width})^2$. 0.5T, $n=6$ myocytes; 1.5T, $n=8$ myocytes. **B:** Changes in cell

thickness and width during anisotonic challenges. Myocyte images were digitized at 10 s intervals, except for measurements of cell thickness at 6, 15, and 22 min (Ba). The thickness/width ratio (T/W) in Bb was measured from each myocyte during sequential superfusion with 1T, 0.5T, and 1T solutions. $n=11-33$ myocytes.

tion caused a rapid and reversible cell swelling or shrinkage. Exposure to hyposmotic 0.5T solution for 10 min significantly increased cell width to 1.17 ± 0.01 ($n=6$), and relative cell volume calculated as $\text{length} \times (\text{width})^2$ (lw^2) was 1.38 ± 0.07 ($p < 0.05$ vs. 1T, $n=6$) (Fig. 2Aa). In contrast, hyperosmotic 1.5T solution significantly decreased cell width to 0.91 ± 0.01 and lw^2 volume to 0.81 ± 0.02 ($n=8$) after 10 min (Fig. 2Ab). Under both conditions, there were no significant changes in cell length (1.01 ± 0.003 in 0.5T, $n=6$; 0.96 ± 0.01 in 1.5T, $n=8$), suggesting the presence of longitudinal cellular load-bearing structures [14–16]. These new steady-states were attained in 2–5 min, and there were no later volume regulatory responses.

An estimation of cell volume by $\text{length} \times (\text{width})^2$ assumes that changes in cell width and thickness under anisotonic conditions are proportional. The validity of the assumption was verified by measuring width and thickness from individual cells during se-

quential superfusion with 1T, 2.2T, and 0.5T solutions. An example experiment depicted in Fig. 2Ba indicates that a myocyte narrowed and flattened to ~ 0.82 in 2.2T solution and widened and thickened to ~ 1.18 in 0.5T solution. A summary of the results in Fig. 2Bb indicates that neither swelling nor shrinkage significantly changed the thickness/width ratio (0.65 ± 0.03 in 0.5T, $n=22$; 0.66 ± 0.05 in 1.5T, $n=13$; 0.64 ± 0.06 in 2.2T, $n=11$) from that in 1T solution (0.65 ± 0.03 , $n=33$).

Osmometric behavior of myocytes. Myocytes were equilibrated in 1T solution, then superfused for 10 min with one of the anisotonic solutions. The steady-state data in Fig. 3A indicate that changes in cell length were modest compared to reductions in cell width, and that changes in cell thickness closely paralleled the changes in cell width. Relative lw^2 volumes of 1.38 ± 0.02 ($n=16$), 1.19 ± 0.02 ($n=7$), 0.77 ± 0.02 ($n=13$), 0.66 ± 0.03 ($n=10$), and

Fig. 3. Osmotically induced changes in myocyte parameters. **A:** Summary of changes in cell dimensions and calculated (*lwt* and *lw*²) volumes after 10 min in anisotonic solutions. *n*=7–16 myocytes. All data, except cell length under hypotonic conditions, are significantly different (*p*<0.001) from isosmotic values. **B:** A van't Hoff plot of relative *lw*² volume vs. the reciprocal of the relative osmolality of bathing solution. The straight line is a linear regression fit to mean data (closed squares, *n*=5–13 myocytes): $V_{rel} = \{(1 - V_b)/\pi_{rel}\} + V_b = (0.65/\pi_{rel}) + 0.35$, where V_{rel} is the relative cell volume, π_{rel} is the relative solution osmolality, and the intercept with the volume axis 0.35 (arrow) is an estimate of the osmotically inactive fraction V_b . Also included are data from earlier studies on rat [15, 16], rabbit [17], and cat [16] ventricular myocytes. The dotted line illustrates the theoretical relationship for perfect osmometer behavior. The inset depicts van't Hoff data from myocytes treated with hyperosmotic Cl⁻-free+10 μ M ouabain solutions (*n*=10–12 myocytes); the open arrow indicates the volume axis intercept of 0.22.

0.58 ± 0.02 (*n*=9) in 0.5T, 0.75T, 1.5T, 2.2T, and 2.8T solutions, respectively, were therefore nearly identical to those calculated with independent parameters (relative *lwt* volume: 1.41 ± 0.04 , 1.19 ± 0.02 , 0.78 ± 0.03 , 0.66 ± 0.02 , and 0.57 ± 0.02 in the respective solutions).

Figure 3B depicts a van't Hoff plot of the *lw*² volume against the reciprocal of relative osmolality. One notable feature is that the myocyte volume (filled squares) did not change to the degree expected of a perfect osmometer (dotted line with intercept at zero

cell volume), and an extrapolation of the regression line to the cell volume axis suggests a limiting value of 0.35 (arrow). Another feature is that the volume data obtained under 0.75T through 4T conditions were described by the van't Hoff relationship (see Fig. 3 legend for van't Hoff equation), whereas the 0.5T data (rightmost square) was slightly smaller than predicted by the relationship. The data from earlier volumetric studies on rat [15, 16], rabbit [17], and cat [16] ventricular myocytes also exhibited similar trends (open symbols).

A common explanation for the deviation from the theoretical van't Hoff relationship is that a fraction of the cell volume is osmotically inactive [15, 17]. To obtain additional information on this point, another group of myocytes were subjected to hyperosmotic challenges after inhibiting osmolyte fluxes via Cl⁻ transport and Na⁺-K⁺ pumping, two processes that have been expected to contribute to cardiac cell volume regulation [17]. Under isosmotic conditions, a 10-min treatment with Cl⁻-free solution that contained 10 μ M ouabain caused no significant change in relative cell volume (1.00 ± 0.02 , *n*=33). Subsequent exposures to hyperosmotic Cl⁻-free+ouabain mixtures caused larger shrinkage than that caused by the standard challenges, resulting in a steeper regression line with the volume intercept of 0.22 (Fig. 3B, inset). In summary, the van't Hoff relationship is helpful in interpolating between the measured points, but the volume intercept per se has little real meaning if compensatory ion fluxes occur during the measurement period. Because there are no clear-cut prescriptions to handle the nonzero volume intercept, the empirical value of 0.35 is used for P_f calculation (see Eq. 2), as in earlier study by others (0.34 for rabbit ventricular myocytes [18]).

Water permeability of myocyte membranes

Figure 4 shows an analysis of the time course of osmotic swelling with data from a representative myocyte exposed to a 0.5T solution. Upon a switch from 1T to 0.5T solution, the myocyte rapidly swelled to a new steady volume of 1.38, with the half-time (*t*_{1/2}) of ~35 s (corrected for the lag time because of solution exchange). The first derivative of relative volume (dV_{rel}/dt) reached a maximum after 40 s, and the osmotic gradient ($\Delta\pi$) gradually declined during 0.5T superfusion. The permeability coefficient P_f calculated from the peak (40 s) value of $(dV_{rel}/dt)/\Delta\pi$ by the Eq. 1 (see METHODS) was $21.5 \mu\text{m} \cdot \text{s}^{-1}$. The data from similar experiments on 0.5T-induced swelling gave the averaged P_f of $23.6 \pm 1.9 \mu\text{m} \cdot \text{s}^{-1}$ (*n*=9) at 35°C.

Fig. 4. Analysis of the time course of osmotic cell swelling using data from a representative myocyte. The top panel shows example images of the middle portion of the myocyte demonstrating an increase in cell width during 5 min superfusion with 0.5T solution (35°C). Sequential cell images were digitized at 10 s intervals to obtain relative lw^2 volume (V_{rel}), its first derivative (dV_{rel}/dt), and the osmotic gradient ($\Delta\pi$). The water permeability coefficient (P_f) was calculated from the peak (40 s) value of $(dV_{rel}/dt)/\Delta\pi$ by Eqs. 1 and 2 (see METHODS).

Water permeation across biological membranes is dependent on temperature [2–4]. As depicted in Fig. 5A, the rate of cell swelling and shrinkage was slower at lower temperatures: $t_{1/2}$ increased from 36.3 ± 3.9 s ($n=9$) at 35°C to 73.4 ± 9.1 s ($n=8$) at 22°C, and 112.1 ± 13.4 s ($n=8$) at 11°C for 0.5T-induced swelling, and from 8.7 ± 0.7 s ($n=5$) at 35°C to 20.1 ± 5.0 s ($n=7$) at 11°C for 2.2T-induced shrinkage. P_f obtained from these challenges was 23.6 ± 1.9 ($n=9$), 12.6 ± 3.3 ($n=8$), and 8.0 ± 1.5 ($n=8$) $\mu\text{m} \cdot \text{s}^{-1}$ for swelling at 35, 22, and 11°C, respectively, and 19.8 ± 1.4 ($n=5$) and 7.2 ± 1.2 ($n=7$) $\mu\text{m} \cdot \text{s}^{-1}$ for shrinkage at 35 and 11°C, respectively. P_f calculated for cell shrinkage in response to greater osmotic gradient (4T) was 11.9 ± 1.0 ($n=5$) and 7.6 ± 0.2 s ($n=4$) $\mu\text{m} \cdot \text{s}^{-1}$ at 22 and 11°C, respectively.

Fig. 5. Temperature dependence of osmotic swelling and shrinkage. **A:** Time course of cell swelling in 0.5T solution (Aa) and half-time ($t_{1/2}$) (Ab) at 11, 22, and 35°C. The $t_{1/2}$ was measured by reference to quasi-steady-state (10 min) volume. **B:** Arrhenius plot of water permeability coefficient (P_f). The activation energy (E_a) given by the slope of the plot was 3.9, 3.7, and 3.4 kcal·mol $^{-1}$ under 0.5T, 2.2T, and 4T conditions, respectively. $n=4$ –9 myocytes.

The Arrhenius activation energy E_a provides a measure of the energy barrier to water movement across a membrane and is determined from the slope of a semilogarithmic plot of P_f vs. $1/\text{temperature}$ (Fig. 5B). E_a measured between 11 and 35°C was 3.9 kcal·mol $^{-1}$ for swelling in 0.5T solution, 3.7 kcal·mol $^{-1}$ for shrinkage in 2.2T solution, and 3.4 kcal·mol $^{-1}$ for shrinkage in 4T solution. These values, which are equivalent to E_a for the self-diffusion of water in bulk solution (~ 4 kcal·mol $^{-1}$), indicate that the temperature dependence of water transport across guinea pig heart cell membranes is relatively low in comparison with diffusion of water through lipid membranes (> 10 kcal·mol $^{-1}$) [2–4].

Hg $^{2+}$ inhibition of osmotic cell swelling

The weak temperature dependence of myocyte P_f suggests that water transverse the sarcolemma through water-filled pores. To obtain additional information on this point, the time course of osmotic swelling was investigated in myocytes treated with Hg $^{2+}$, an inhibitor of pore-mediated water transport across cell membranes [3, 4, 19]. The results shown in

Fig. 6. Hg²⁺ inhibition of osmotic swelling and P_f in 0.5T solution. **A:** Effects of 0.1 mM HgCl₂ and 10 mM dithiothreitol (DTT) on the time course of swelling (Aa), quasi-steady-state (10 min) volume (Ab), and P_f (Ac) at 35°C. Myocytes were exposed to 0.5T, 0.5T+DTT, 0.5T+Hg²⁺, and 0.5T+Hg²⁺+DTT solutions. $n=5-9$ myocytes. * $p<0.05$ and ** $p<0.001$ vs. pre-0.5T; † $p<0.001$ vs.

control 0.5T. **B:** Temperature dependence of Hg²⁺ actions on the time course of swelling (Ba) and Arrhenius plot of P_f (Bb). Data were obtained from myocytes exposed to 0.5T solution with and without 0.1 mM Hg²⁺ at 11–35°C. The activation energy (E_a) was 3.9 and 12.3 kcal·mol⁻¹ in control and Hg²⁺-treated cells, respectively. $n=5-9$ myocytes.

Fig. 6Aa indicate that treatment with 0.1 mM HgCl₂ inhibited cell swelling during 0.5T+Hg²⁺ superfusion. Relative cell volume after 10 min in 0.5T solution was significantly smaller in Hg²⁺-treated myocytes (1.10 ± 0.02 , $n=5$) than in non-Hg²⁺-treated myocytes (1.38 ± 0.02 , $n=9$) (Fig. 6B). The calculated P_f in Hg²⁺-treated myocytes ($2.9 \pm 0.2 \mu\text{m} \cdot \text{s}^{-1}$, $n=5$) was ~8 times smaller than that of non-Hg²⁺-treated myocytes ($23.6 \pm 1.9 \mu\text{m} \cdot \text{s}^{-1}$, $n=9$) at 35°C.

Mercurials are sulfhydryl-oxidizing reagents, and their biological actions can be antagonized by sulfhydryl-reducing agents [3, 4, 19]. As indicated by the closed symbols and bars in Fig. 6A, the inhibition of cell swelling by 0.5T+Hg²⁺ treatment was reversed by the reducing agent dithiothreitol (DTT, 10 mM), whereas DTT had little effect on swelling in the absence of Hg²⁺. P_f in myocytes exposed to 0.5T+Hg²⁺+DTT solution ($20.0 \pm 1.3 \mu\text{m} \cdot \text{s}^{-1}$, $n=6$)

was not significantly different than measured in non-treated myocytes (Fig. 2Ac). These results suggest that the Hg²⁺ inhibition of P_f is due to binding of the metal to sulfhydryl groups in proteins associated with water-traversing membrane pores.

If the primary pathway for osmotic water movement is the membrane pores, water flow remaining after blockade of the pores should exhibit higher temperature dependence, i.e., larger E_a [18]. To test this possibility, P_f was calculated from the time course of 0.5T-induced swelling in 0.1 mM Hg²⁺-treated myocytes at 35 and 11°C (Fig. 6Ba). E_a measured between 11 and 35°C was 12.3 kcal·mol⁻¹ (Fig. 6Bb, closed symbols); this value is ~3-fold greater than that for non-Hg²⁺-treated myocytes (3.9 kcal·mol⁻¹; Fig. 6Bb, open symbols) and is in the range of values for lipid membranes not containing functioning water channels (8–15 kcal·mol⁻¹) [1].

DISCUSSION

The purpose of this study was to evaluate osmotically induced volume change and sarcolemmal water permeability of guinea pig ventricular myocytes. The results demonstrate osmometric properties of heart cells: (1) the unchanged cross-sectional geometry and the differential compliance in the radial and axial directions; (2) a linear van't Hoff behavior predicting the $\sim 35\%$ osmotically dead volume; (3) the lack of evidence for a short-term volume compensation; (4) water permeability 5–20 times smaller than that of water-transporting tissues; and (5) the low E_a and the sulfhydryl-related modifications of P_f suggesting a channel-mediated water transport.

Osmotic cell swelling and shrinkage. Estimation of cross-sectional geometry is central to the calculation of myocyte volume. On the assumption that a myocyte is a rectangular parallelepiped, the mean volume of the myocyte was 43 pl; the calculated volumes are 34 pl for an elliptical cylinder [14, 20], 54 pl for a circular cylinder [15, 21, 22], and 68 pl for a rectangular parallelepiped with equal width and thickness [17]. The last two are clearly erroneous, since guinea pig ventricular myocytes are brick-shaped with the thickness/width ratio of ~ 0.65 , and their cross-sectional geometry is unchanged after osmotic swelling and shrinkage.

The assumption on myocyte geometry has much importance to the determination of P_f because the surface/volume ratio is inversely proportional to P_f (see Eq. 1). The surface/volume ratio calculated for a brick-shaped myocyte with 120 μm length, 23 μm width, and 15 μm thickness is $0.24 \mu\text{m}^2 \cdot \mu\text{m}^{-3}$. If the cell surface area is estimated from membrane capacitance (C_m) of guinea pig ventricular myocytes ($152 \pm 5 \text{ pF}$, $n=80$) and a specific C_m of $1 \mu\text{F} \cdot \text{cm}^{-2}$ [23], the ratio is $0.37 \mu\text{m}^2 \cdot \mu\text{m}^{-3}$, and therefore P_f is ~ 0.7 times the values obtained by the standard calculation. The divergence may reflect the presence of membrane folding, invaginations, and T tubules [24], but these structures cannot be resolved with the present microscope arrangement.

Earlier studies on isolated cat [16], ferret [14], rabbit [17], and rat [14–16] ventricular myocytes have shown that changes in cell width under anisosmotic conditions were larger than the changes in cell length. Our measurements indicate that changes in cell width and thickness are proportional and much larger than the changes in cell length. This probably reflects directional differences in the elastic characteristics of the internal load-bearing structures (sarcolemma, myofilaments, cytoskeleton [14–16]), i.e., the compliance

of a myocyte in the radial direction is uniform, but it is greater than its compliance in the longitudinal direction.

Changes in cell volume under anisosmotic conditions were less than expected from the van't Hoff relationship for the cells exhibiting perfect osmometric behavior. The volume intercept on the van't Hoff plot suggests that guinea pig ventricular myocytes have an apparent osmotically inactive fraction of 35%. This estimate is in agreement with the values reported for rat (18%) [15], cat (23%, estimated from Tanaka *et al.*) [16], and rabbit (34%) [17] ventricular myocytes. The indication of an inactive volume may mean that it is fixed, as in a cellular compartment; the volume occupied by nonaqueous materials (e.g., cell proteins, solute, membranous structures) and protein-bound water could behave as an osmotically dead space [15, 17], but it could also reflect a transmembrane redistribution of osmolytes in response to anisosmotic stress. There are grounds for expecting that volume-regulatory processes are activated in the myocytes: The hyperosmotic shrinkage of rabbit ventricular myocytes was larger in the presence of Cl^- transport blockers or ouabain [17], and we observed more pronounced shrinkage under Cl^- -free+ouabain conditions (Fig. 3B, inset). When the activity of these compensatory mechanisms is curtailed, guinea-pig cells may approach perfect osmometer status.

Membrane water transport. P_f of guinea pig ventricular myocytes ($\sim 22 \mu\text{m} \cdot \text{s}^{-1}$ at 35°C) is considerably lower than that of specialized water-transporting cells, such as renal tubules ($185\text{--}250 \mu\text{m} \cdot \text{s}^{-1}$) [25, 26] and mammalian erythrocytes ($120\text{--}530 \mu\text{m} \cdot \text{s}^{-1}$) [27, 28] and is in the range of diffusional water permeability across lipid bilayers and liposomes ($2\text{--}50 \mu\text{m} \cdot \text{s}^{-1}$) [1]. However, the observed E_a ($\sim 3.8 \text{ kcal} \cdot \text{mol}^{-1}$) is much lower than the values for lipid membranes without functioning water channels ($8\text{--}15 \text{ kcal} \cdot \text{mol}^{-1}$) [1], and indicates that the energy barrier of water crossing the cardiac sarcolemma is as low as the value for free-water diffusion ($\sim 4 \text{ kcal} \cdot \text{mol}^{-1}$). Furthermore, treatment with Hg^{2+} , a sulfhydryl reagent commonly used to block water channels [4, 19], inhibited P_f by $\sim 90\%$ and increased E_a to $12 \text{ kcal} \cdot \text{mol}^{-1}$. Taken together, these data indicate that water crosses guinea pig myocyte membranes mainly by the water-channel-mediated mechanism.

Suleymanian and Baumgarten [18] measured sarcolemmal hydraulic conductivity (L_p) from rabbit ventricular myocytes and obtained a low value (L_p of $1.2 \times 10^{-10} \text{ l} \cdot \text{N}^{-1} \cdot \text{s}^{-1}$, equivalent to P_f of $16 \mu\text{m} \cdot \text{s}^{-1}$ at 22°C) that is comparable to the guinea pig P_f

($12 \mu\text{m} \cdot \text{s}^{-1}$ at 22°C). However, they obtained higher E_a ($12 \text{ kcal} \cdot \text{mol}^{-1}$) and thereby concluded that the primary route for water crossing cardiac membranes is directly through the lipid bilayers. There are no substantial differences in experimental conditions and data acquisition between their study and ours; data analysis is also unlikely to be a factor because the calculation of L_p from the guinea pig data by using their formula still gives a low E_a ($\sim 3.7 \text{ kcal} \cdot \text{mol}^{-1}$). Thus the divergence is most likely to be due to species difference in the expression of sarcolammal water channels. From the single-channel P_f of aquaporin-1 ($\sim 10^{-13} \text{ cm}^3 \cdot \text{s}^{-1}$) [29] and the Hg^{2+} -inhibited P_f of $\sim 12 \mu\text{m} \cdot \text{s}^{-1}$ at room temperature, a density of aquaporin monomer is estimated to be $120 \mu\text{m}^{-2}$ for guinea pig myocyte membranes. This estimate is roughly an order of magnitude greater than the membrane density of cardiac ionic channels, but much less than the density of aquaporin-1 in erythrocytes ($\sim 2 \times 10^5$ monomers per erythrocyte, corresponding to $\sim 1,500 \mu\text{m}^{-2}$) [3, 29]. If the density of aquaporin in cardiac membranes is $10 \mu\text{m}^{-2}$ rather than $120 \mu\text{m}^{-2}$, it would make a physiologically insignificant contribution to myocyte P_f ($\sim 1 \mu\text{m} \cdot \text{s}^{-1}$).

Unstirred layer effects are another concern in the low myocyte P_f . It is believed that the osmotic water flow sweeps solute toward unstirred layers adjacent to the membrane on one side and away from the membrane on the other [2, 30]. These "sweeping-away" effects act to reduce the osmotic gradient around the cell surface and can lead to an underestimation of P_f . The experimentally observed P_f ($P_{f(\text{obs})}$) is described as $P_{f(\text{obs})} = P_{f(\text{memb})} \exp(-J_v \delta / D_s)$, where $P_{f(\text{memb})}$ is the true membrane P_f , J_v is the water volume flux, δ is the thickness of the unstirred layer, and D_s is the diffusion coefficient of the osmolyte [2]. J_v was calculated from the time course of cell swelling and shrinkage, δ from $\delta = (D_s \cdot t_{0.9})^{0.5}$ where $t_{0.9}$ is the time for the concentration to reach 90% of its steady-state value, and D_s from the concentration-weighted average of the diffusion coefficient (25°C) of NaCl ($1.612 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$) and sucrose ($0.523 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$) in 0.5T, 2.2T, and 4T solutions. The calculated $P_{f(\text{memb})}$ was 12.7, 11.9, and $11.9 \mu\text{m} \cdot \text{s}^{-1}$ at 22°C in 0.5T, 2.2T, and 4T solutions, respectively (vs. $P_{f(\text{obs})}$ of 12.6, 11.7, and $11.9 \mu\text{m} \cdot \text{s}^{-1}$, respectively; see RESULTS); the differences between $P_{f(\text{memb})}$ and $P_{f(\text{obs})}$ are $<2\%$ and may be negligible, as estimated earlier for rabbit ventricular myocytes ($<3\%$) [18].

Physiological and pathophysiological implications. Renal tubules, for example, require high water permeability for the urine-concentrating mechanism [3, 31]. In contrast, the low water perme-

ability of cardiac membranes seems to be less important in physiological cell volume regulation because heart cells normally do not experience significant osmotic perturbations. However, it may have functional significance under pathophysiological conditions by restricting the rapidity of osmotic cell swelling and shrinkage. For example, cardiac ischemia and reperfusion cause cell swelling and membrane disruption [32]; the intracoronary injection of hyperosmotic radiocontrast medium results in side effects on electromechanical activities [33]. Transient osmotic disturbances underlying these events would be exacerbated if the water permeability was high. Recently, the regulation of water channel function is thought to be a potential therapeutic strategy for a variety of cardiovascular disorders, including congestive heart failure, hypertension, lung edema, and brain swelling [5, 34]. Further investigation is warranted because abnormalities in osmotic and volume homeostasis can have consequences for heart cell function.

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