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Effects of Anisosmotic Medium on Cell Volume, Transmembrane Potential and Intracellular K^+ Activity in Mouse Hepatocytes

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Summary. Mouse hepatocytes in primary monolayer culture (4 hr) were exposed for 10 min at 37°C to anisosmotic medium of altered NaCl concentration. Hepatocytes maintained constant relative cell volume (experimental volume/control volume) as a function of external medium relative osmolality (control mOsm/experimental mOsm) ranging from 0.8 to 1.5. In contrast, the relative cell volume fit a predicted Boyle-Van't Hoff plot when the experiment was done at 4°C. Mouse liver slices were used for electrophysiologic studies, in which hepatocyte transmembrane potential (V_m) and intracellular K^+ activity (a^i_K) were recorded continuously by open-tip and liquid ion-exchanger ion-sensitive glass microelectrodes, respectively. Liver slices were superfused with control and then with anisosmotic medium of altered NaCl concentration. V_m increased (hyperpolarized) with hypoosmotic medium and decreased (depolarized) with hyperosmotic medium, and ln [10(experimental V_m/control V_m)] was a linear function of relative osmolality (control mOsm/experimental mOsm) in the range 0.8–1.5. The a^i_K did not change when medium osmolality was decreased 40–70 mOsm from control of 280 mOsm. Similar hypoosmotic stress in the presence of either 60 mM K^+ or 1 mM quinine HCl or at 27°C resulted in no change in V_m compared with a 20-mV increase in V_m without the added agents or at 37°C. We conclude that mouse hepatocytes maintain their volume and a^i_K in response to anisosmotic medium; however, V_m behaves as an osmometer under these conditions. Also, increases in V_m by hypoosmotic stress were abolished by conditions or agents that inhibit K^+ conductance.

Key Words hepatocyte · cell volume · K^+ conductance · temperature · quinine HCl · intracellular K^+ activity

Introduction

Hepatic function comprises various metabolic reactions, including hormonal regulation of hepatocyte organic solute content. For example, catecholamine-stimulated hepatic glycogenolysis increases intracellular concentrations of osmotically active glucose molecules, whose intracellular concentrations must exceed extracellular concentrations for net, passive efflux to occur. For this to happen without cell swelling requires regulation of cell volume; however, the mechanisms are unknown. Certain volume control mechanisms reverse cell swelling, such as regulatory volume decreases associated with increases in cell K^+ efflux (Kregenow, 1981; Siebens, 1985). Consequently, it is consistent that hepatic K^+ efflux precedes glucose efflux following hormonal stimulation of glycogenolysis (Shoemaker & Elwyn, 1969). Similar cell K^+ efflux has been invoked as a means for cell volume regulation during hepatocyte amino acid transport (Kristensen, 1986).

Hepatocyte alanine transport occurs by secondary active transport systems (systems A and ASC) coupled to the transmembrane electrochemical Na^+ gradient (Kristensen, 1980, Kilberg, 1982). Alanine and Na^+ accumulation leads to cell swelling and corresponding increases in K^+ efflux, which presumably constitutes part of regulatory volume decrease (Bakker-Grunwald, 1983; Kristensen & Folke, 1984). Hepatocyte alanine transport also is rheogenic (Fitz & Scharschmidt, 1987*a*; Wondergem & Castillo, 1987). In mammalian hepatocytes, transmembrane potential, V_m, decreases with alanine transport, but with alanine still present externally, V_m repolarizes, often to values more electronegative than control V_m. Wondergem and Castillo (1987) have reported that this repolarization is blocked by quinine HCl, as is the alanine-induced K^+ efflux (Kristensen & Folke, 1984).

We postulate that repolarization of hepatocyte V_m during rheogenic, alanine transport may be important for cell mechanisms regulating hepatocyte volume. As a preliminary step toward exploring these cell mechanisms, we have conducted experiments to determine the effects of anisosmotic, external medium on hepatocyte V_m, intracellular K^+ activity, a^i_K, and relative cell volume.

Materials and Methods

Materials and Animals

All chemicals and reagents were purchased from either Fisher or Sigma. Adult mice (male or female) of ICR strain were fasted 18–24 hr before experiments.

Liver Slice Preparation and Superfusion with Krebs Medium

Mice were killed by cervical dislocation, and the left-lateral or medium lobe of the liver was removed quickly and placed on gauze moistened with 0.9% NaCl. A glass microscope slide was pressed gently onto the lobe to keep it from moving while a slice of ~1 mm thick was made by using a razor blade clamped in a hemostat. A slice of ~5 × 5 mm surface area was placed into an acrylic chamber (2 ml vol), and it was held in place by a small steel washer. This ensured that microelectrode impalements were always of cells on the encapsulated, uncut surface of the slice.

Liver slices were superfused with Krebs physiological salt medium containing (in mM): 103 NaCl, 4.7 KCl, 2.56 $CaCl_2$, 1.3 $MgCl_2$, 25 $NaHCO_3$, 1.15 NaH_2PO_4, 2.8 glucose, 4.9 Na-pyruvate, 4.9 Na-glutamate, 2.7 Na-fumarate equilibrated with 95% O_2-5% CO_2. Temperature at the liver slice was monitored continuously with a thermistor (Yellow Springs Instrument, Yellow Springs, OH), and it was maintained at 37°C by passing medium first through a Graham condenser and then through the tissue bath. This enabled countercurrent heat exchange to medium from water circulating through the condenser jacket and heater pump. Flow rate was 6–7 ml/min, and the period for fluid exchange in the bath was 26 ± 2.5 sec (n = 7). In most experiments, medium osmolality was changed by decreasing (hypoosmotic) or increasing (hyperosmotic) NaCl concentrations. For some experiments, hyperosmotic conditions were created by adding sucrose to the medium. Osmolality of all solutions was measured directly by freezing point depression (Precision Systems, Sudbury, MA).

Fabrication of Open-Tip and Ion-Sensitive Microelectrodes

Microfilament glass capillaries (1.2-mm o.d., 0.68-mm i.d., A-M Systems, Everett, WA) were cleaned by boiling them for 15 min in 500 ml of distilled water plus three drops of liquid detergent (Liquinox). This was followed by a 1-hr tap water rinse, boiling for 15 min in distilled water, and drying at 90°C in a forced-air oven. Micropipettes were drawn in a horizontal puller (Industrial Scientific, Ridgewood, NY) from the microfilament capillaries. Open-tip microelectrodes were filled with 0.5 M KCl.

Double-barreled ion-sensitive microelectrodes were prepared from fiber-filled, borosilicate double capillaries (1.2-mm o.d., 0.6-mm i.d.; A-M Systems, Everett, WA) cleaned as described above. These were pulled in a vertical puller (700D, David Kopf Instruments, Tujunga, CA) and had tip diameters of ~1 μM. The tip of one barrel was filled with a 1-mm column of silicone/acetone (0.1%, DC1107, Dow Corning, Midland, MI) and baked on a hot plate at 300°C for 30 min. The tip of the siliconized barrel was filled with a 1-mm column of K^+ exchanger (Corning 477317) and backfilled with 0.5 M KCL. The reference barrel was filled with 0.5 M NaCl. The K^+-selective microelectrode was calibrated (after impalements) in pure solutions of KCl within the range of physiologic concentrations and at room temperature, since calibration curves do not vary between 22 and 37°C (Edelman et al., 1978).

All microelectrodes were connected by Ag-AgCl half-cells to a high input impedance ($>10^{14}$ Ω) preamplifier with unit gain (515L Analogue Devices, Norwood MA). Reference electrodes consisted of a Ag-AgCl half-cell connected to the tissue chamber by an agar (4% in Krebs medium). Voltages were recorded on digital voltmeters (Keithly), a storage oscilloscope (Tektronix), a Grass polygraph, and a strip-chart recorder (Western Graphtec).

Calibration of Ion-Sensitive Microelectrodes

Ion-sensitive microelectrodes were calibrated in ionic solutions covering the physiologic range. Ion activities in these calibration solutions were computed according to the Debye-Hückel equation as modified by Armstrong, Byrd and Hamang (1973) and according to data of Conway (1969). K^+-selective microelectrodes were calibrated in 200, 100, 50 and 10 mM KCl solutions. For five electrode calibrations, the slopes of electrode voltage *vs.* K^+ activity ranged from 50 to 63 mV/10-fold change in K^+ activity, with a mean ± SE of 60 ± 1.2. Microelectrode selectivities for K^+ over Na^+ were computed from V measurements in 0.1 M salt concentrations and averaged 37 ± 10.3. Since microelectrode selectivities vary with ion concentration (Armstrong & Garcia-Diaz, 1980; Edelman et al., 1978), and since our experiments below involved changes in NaCl concentration in the bath, a^i_K was calculated by direct interpolation from the calibration curve rather than by the Nicolsky equation. The regression of the K-selective microelectrode potential, V, *vs.* log a_K in pure KCl is

$$V_K = S \cdot \log a_K + b. \quad (1)$$

Consequently, a^i_K is computed according to

$$a^i_K = 10(V_K - V_m - b)/S \quad (2)$$

where V_K and V_m are potentials recorded in the intracellular space with K-selective microelectrodes and open-tip microelectrodes, S and b are slope and intercept of the regression line [Eq. (1)].

Intracellular Measurements with Microelectrodes

Criteria for valid micropipette impalements of liver cells were (i) rapid deflection of the voltage trace on advancing a microelectrode into the tissue, (ii) an intracellular voltage trace that was stable within 2 mV for at least 1 min, (iii) return of the voltage trace to within 2 mV of base line when a microelectrode was withdrawn from a cell. Resistance of open-tip microelectrodes was measured intermittently during impalements by passing 0.5 nA of current (300-msec duration) through the recording microelectrode. Microelectrode resistance is 10^2 greater than input resistance in mouse liver slices (Graf & Petersen, 1978), which made it possible to measure microelectrode resistance throughout the impalements. We regarded irreversible increases in microelectrode resistance of 10 MΩ or greater to result from clog-

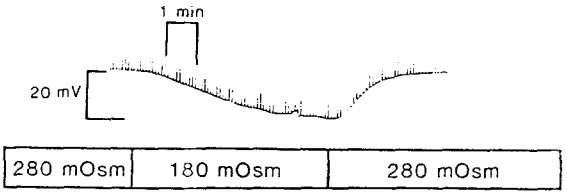

Fig. 1. Effect of hypoosmotic salt solution on V_m measured in a mouse liver slice. Hypoosmotic conditions were created by switching to a physiological salt solution containing a lower NaCl concentration. Vertical deflections result from 0.5 nA intermittent current. Downward movement of the voltage trace indicates hyperpolarization

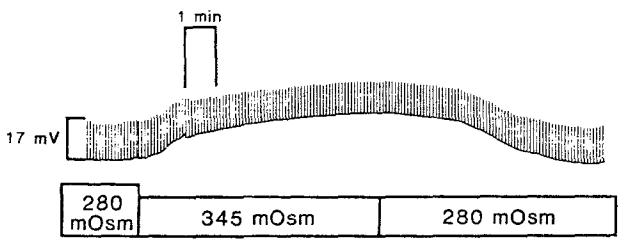

Fig. 2. Effect of hyperosmotic salt solution on V_m measured in a mouse liver slice. Hyperosmotic conditions were created by switching to a physiological salt solution containing a higher NaCl concentration. Vertical deflections result from 0.5 nA intermittent current. Upward movement of the voltage trace indicates depolarization

ging of the tip, and these recordings were not included in the data. Current was not passed during recordings with the double-barreled K-selective microelectrodes, because of the high resistances of the K-selective electrode (>10 GΩ). Microelectrodes were repositioned after every impalement to ensure that consecutive impalements were not of the same cell. Stable intracellular recording of V_m were maintained routinely for 15–30 min while effects of osmotic changes on V_m were obtained. Also, V_m was recorded continuously when external K^+ concentration was increased above control values by replacing external Na^+ with an equivalent amount of K^+.

Hepatocyte Isolation and Primary Culture

Adult, female mice were fasted 12–18 hr prior to isolating hepatocytes by the technique of Klaunig et al. (1981). Liver was perfused *in situ* (backward through the thoracic inferior vena cava with perfusate draining through the sectioned portal vein and not recirculated) for 5 min at 10 ml/min with Krebs physiologic salt medium, minus $CaCl_2$ and $MgCl_2$, and equilibrated with 9% O_2–5% CO_2 at 37°C. It was then perfused for 10–15 min with complete Krebs medium plus 0.5 mg/ml collagenase.

Cell suspensions were prepared by mechanical dispersion of the enzyme-dissociated liver. Hepatocytes were obtained by filtering cell suspensions through 253 and 63 μM nylon mesh screens, respectively, and then centrifuging the filtrates at 50 × *g* for 7 min. The pellets of hepatocytes were resuspended, washed and recentrifuged. They were suspended (approx. 10^6 cell/ml) in Eagle minimal essential medium containing Hanks salts, 1 g/liter bovine serum albumin, 1 mU/ml insulin and 50 μg/ml gentamicin sulfate. The hepatocyte suspension was dispensed in 3 ml aliquots into 60 mm, collagen-coated (rat tail-tendon) culture plates containing plastic coverslips and incubated at 37° in air/CO_2 (pH 7.4). Cells attached to the coverslips by 4 hr.

Determination of Relative Hepatocyte Volume

After 4 hr of cell attachment, coverslips were transferred (time zero) to 60-mm dishes containing Krebs physiological salt medium of various osmolalities obtained by altering the concentration of NaCl. ($NaHCO_3$ of these solutions was reduced to 20 mM and 5 mM HEPES added. The pH was adjusted to 7.4 with 1 N HCl. Osmolalities were measured as above by freezing point depression.) The salt medium also contained 0.1 μCi/ml ^{14}C-polyethyleneglycol. After 10 min incubation, the coverslips were removed and the bottom-sides blotted quickly onto an absorbant pad. They were placed onto dry ice for approximately 1 min until the cells were frozen. Freezing minimized evaporation water loss while the cells and coverslips were weighed on an electrobalance (Cahn). The coverslips were dryed in a forced-air oven at 90°C for 12 hr, and then they were reweighed. Total water was the difference between wet and dry weights. The cells were solubilized in 0.2 N KOH and scraped from the coverslips along with residual ^{14}C-polyethyleneglycol. An aliquot (0.5 ml) of the scrapings was neutralized with 0.4 N acetate, and the radioactivity was determined by liquid scintillation spectrometry. Total extracellular water on each coverslip was computed at each osmolality by dividing cpm/coverslip by the cpm/μl of salt solution at each osmolality. Intracellular H_2O = total H_2O-extracellular H_2O. The μl of intracellular H_2O for each coverslip was divided by the mg of protein on the coverslip as determined by the method of Lowry et al. (1951). Relative cell volumes were computed by (μl cell H_2O · mg protein^{-1}, experimental)/(μl cell H_2O · mg protein^{-1}, control). Control solution was defined as the osmolality of unmodified Krebs physiologic salt solution.

Results

Effects of Altered External Osmolality on the Transmembrane Potential, V_m, of Mouse Hepatocytes

Reducing osmolality of the external medium from 280 to 180 mOsm, by reducing medium NaCl, resulted in a slow, 20-mV increase (hyperpolarization) of V_m over 5 min until a steady state was achieved (Fig. 1). V_m returned to control value approximately 2 min after switching back to control medium. Conversely, V_m decreased (depolarized) 17 mV, to a new steady state, by 5 min after increasing external osmolality from 280 to 345 mOsm with added NaCl (Fig. 2). V_m returned to control value approximately 6 min after switching back to control medium (Fig. 2).

V_m behaved as an osmometer in the range of 385 to 182 mOsm. Results of 61 measurements similar

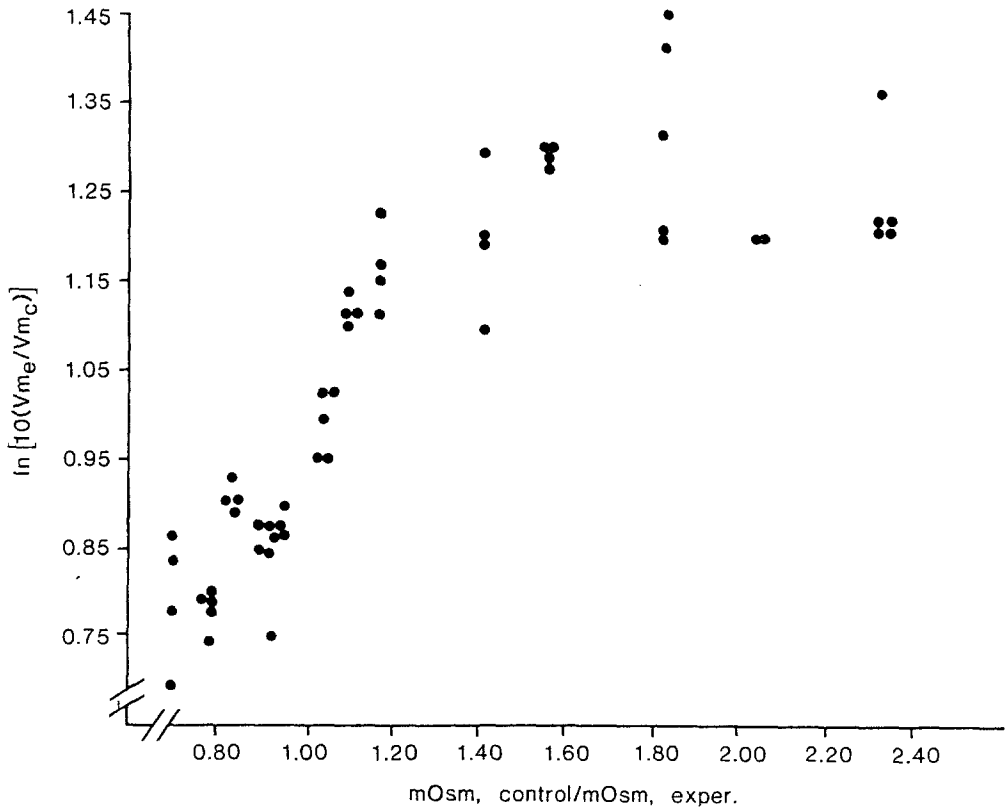

Fig. 3. A plot of the ratios of steady-state V_m *versus* the ratios of extracellular osmolality. Steady-state V_m were obtained under control and various experimental conditions as shown in Figs. 2 and 3. The linear regression coefficient was 0.54 ± 0.034 ($P < 0.001$) for ordinate values in the range 0.8–1.5 on the abscissa ($n = 49$). The correlation coefficient was 0.91. Total determinations for the entire plot was 61

to those shown in Figs. 1 and 2 are summarized in Fig. 3. Here, ratio of steady-state V_m, ln [10(experimental V_m/control V_m)], is plotted as a function of relative osmolalities, control mOsm/experimental mOsm. Experimental osmolalities ranged from 112 to 385 mOsm, compared with an average 274 mOsm for control. Ln [$10(V_{me}/V_{mc})$] varied linearly with the ratio of osmolarities, (control mOsm/experimental mOsm), in the abscissa range from 0.8–1.5 (Fig. 3). The regression coefficient, determined by the least squares computation, was 0.54 ± 0.034 (SE; $n = 49$), $P < 0.001$. The correlation coefficient was 0.91. Values on the ordinate corresponding to values on the abscissa greater than 1.5 were not included, because visual inspection indicated a plateau had been reached. This means that V_m increased no further by extending hypoosmolarity below 182 mOsm.

It is possible that changes of hepatocyte V_m with anisosmotic medium are not associated with osmotic stress but resulted simply from changes in external NaCl and, thereby, Na conductance, gNa. We conducted two experiments to test this. First, hyperosmotic conditions were created by switching to a medium where sucrose was added to increase osmolality 21 mOsm and NaCl was kept constant. It has been shown recently that sucrose does not permeate the hepatocyte plasma membrane (Alpini et al., 1986). Accordingly, V_m decreased 12 mV with hyperosmotic conditions created by added sucrose (*not shown*). Second, external NaCl concentration was reduced 40 mM and replaced with an amount of sucrose so as to make the experimental solution isosmotic with the control solution. We expected V_m to increase if the increases in V_m with hypoosmotic conditions resulted solely from the decrease in NaCl. In contrast, switching from control to experimental solution (low NaCl) decreased V_m 13 mV (*not shown*).

Effects of Changes in External Medium Osmolality on Hepatocyte Membrane K^+ Permeability and Intracellular K^+ Activity, a^i_K

K^+ is the predominent intracellular cation in hepatocytes (Wondergem & Castillo, 1986). Accordingly, membrane K^+ conductance, gK, is thought to be a primary determinant of V_m (Graf et al., 1987),

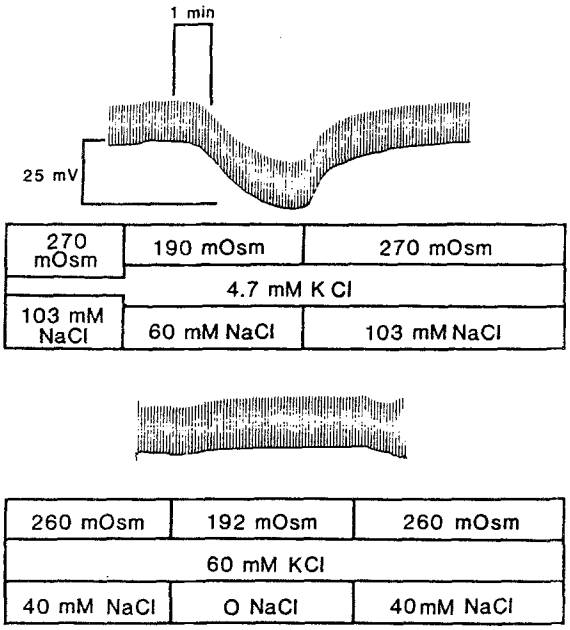

Fig. 4. Top trace shows the effect of hypoosmotic salt solution on hepatocyte V_m (similar to results shown in Fig. 1). Bottom trace shows the effect of hypoosmotic stress on V_m in the same liver slice, after the tissue equilibrated with a salt solution containing 60 mM KCl (KCl substituted for NaCl). The increase in KCl decreased the steady-state V_m 12 mV (*not shown*). Vertical deflections result from 0.5 nA intermittent current. Downward movement of the voltage trace indicates hyperpolarization

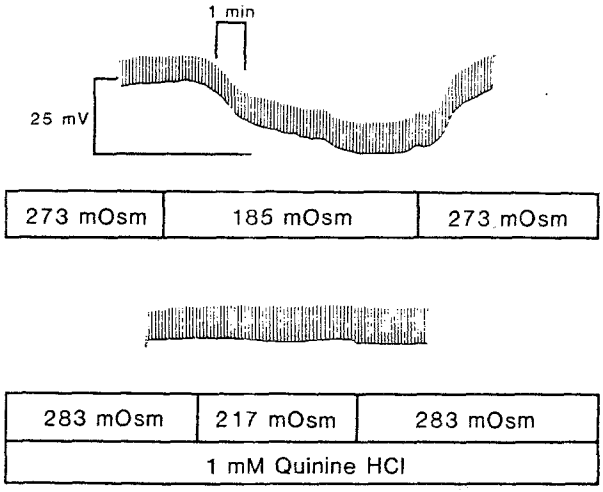

Fig. 5. Top trace shows the effect of hypoosmotic salt solution on hepatocyte V_m (similar to results shown in Fig. 1). Bottom trace shows the effect of hypoosmotic stress on V_m in the same liver slice after the tissue equilibrated with salt solution containing quinine HCl (1 mM). Downward movement of the voltage trace indicates hyperpolarization

and variations in hepatocyte V_m with cell metabolism and membrane transport result from changes in gK and/or intracellular K^+ activity (Wondergem & Harder, 1980; Wondergem, 1982; Chapman & Wondergem, 1984; Wondergem & Castillo, 1986; Henderson, Graf & Boyer, 1986). Consequently, we conducted experiments to determine whether changes in hepatocyte K^+ homeostasis may account, in part, for hepatocyte V_m responding like an osmometer.

We measured the effect of increasing external K^+ concentration on the increases in V_m with hypoosmotic stress. The top trace in Fig. 4 shows a typical response of hepatocyte V_m to hypoosmotic stress. The bottom trace shows the effect of hypoosmotic stress on V_m, in the same liver slice, after the tissue equilibrated with Krebs medium containing 60 mM K^+ (KCl substituted for an equivalent amount of NaCl). The increase in K^+ decreased the steady-state V_m 12–15 mV (*not shown*; Wondergem & Castillo, 1986). Also, hypoosmotic stress did not increase V_m, instead V_m decreased 3mV. This inhibitory effect of 60 mM KCl was reversible. The typical increase in V_m with hypoosmotic stress was restored after normal external $[K^+]$ was restored (*not shown*). These results suggest that increases in V_m with hypoosmotic stress result from increases in membrane gK.

To examine this further, we measured effects of hypoosmotic stress before and after addition quinine, a putative inhibitor of membrane gK (Wondergem & Castillo, 1986). The top trace in Fig. 5 shows a typical increase in hepatocyte V_m in response to hypoosmotic stress. The bottom trace shows the effect of hypoosmotic stress on V_m in the same liver slice, after the tissue equilibrated (10 min) with medium containing quinine HCl (1 mM). Quinine HCl decreases hepatocyte V_m (Wondergem & Castillo, 1986), presumably by decreasing hepatocyte membrane P_K (Burgess, Claret & Jenkinson, 1981). When quinine HCl was added to the medium (1 mM), hypoosmotic stress had no effect on hepatocyte V_m (bottom trace Fig. 5 and Table).

Hepatocyte membrane P_K and V_m are temperature-sensitive (Wondergem & Castillo, 1986). Lowering temperature also inhibited effects of hypoosmotic stress on hepatocyte V_m (Fig. 6). The top trace shows that hypoosmotic stress had no effect on V_m at 27°C; however, while recording V_m in the same cell after increasing temperature to 37°C, hypoosmotic stress increased V_m by 13 mV (bottom trace).

The results above indicate that hypoosmotic stress increases membrane P_K and V_m. Assuming some cell swelling accompanies hypoosmotic stress, we postulated that intracellular K^+ activity, a^i_K, decreases under these conditions. To test this, we measured a^i_K with double-barreled liquid exchanger (Corning 477317) K-sensitive microelec-

Table. Effect of 60 mM K^+, 1 mM quinine HCl, and 10°C temperature decrease on the increase in hepatocyte V_m following hypoosmotic shock[a]

Condition	ΔmOsm (decrease from 274 mOsm)	ΔV_m
Control	79	−20 ± 1.5 (11)
High K^+ (60 mM)	68	3 ± 0.9 (11)[b]
Control	85	−21 ± 2.0 (8)
Quinine HCl (1 mM)	66	−1 ± 0.8 (8)[b]
27°C	66	−1 ± 1.4 (3)
37°C	66	−17 ± 2.2 (3)[b]

[a] Negative sign indicates more electronegative V_m. Parentheses indicates the number of determinations.
[b] Differs from either control or 27°C ($P < 0.05$; Student-Newman-Keuls multiple comparison of means).

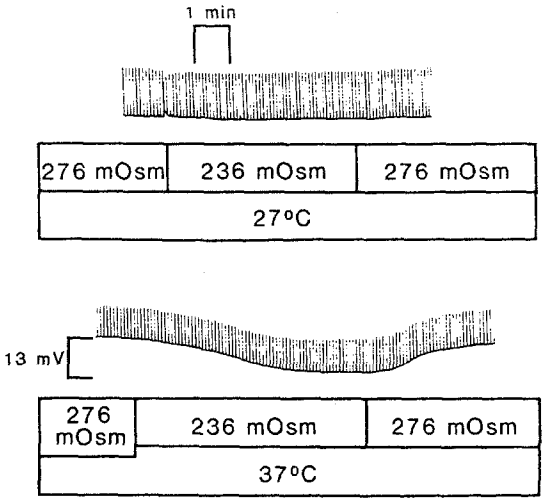

Fig. 6. Top trace shows the effect of hypoosmotic salt solution on hepatocyte V_m in a liver slice maintained at 27°C. Bottom trace shows the effect of hypoosmotic stress on V_m of the same cell after the liver slice had been warmed to 37°C. Warming also increased the steady-state control V_m (*not shown*) according to Wondergem and Castillo (1986). Downward movements of the voltage trace indicates hyperpolarization

trodes. A trace representative of nine measurements is shown in Fig. 7. V_m increased as expected in response to hypoosmotic medium (low NaCl) and returned to control V_m on restoration of control, external osmolality (upper trace). This effect is also measured by V_K, the intracellular voltage of the K-sensitive microelectrode (middle trace). However, $V_m - V_K$, which is proportional to a_K^i did not change during the hypoosmotic stress. The mean a_K^i for control measurements was 73 ± 10 mM ($n = 9$), and the mean a_K^i for paired experimental measurements (hypoosmotic by 40 mM) was 68 ± 10 mM. The difference in mean a_K^i was not significant. Consequently, a_K^i does not decrease with this degree of hypoosmotic stress.

Effect of Osmotic Stress on Hepatocyte Relative Volume

The observation above that hepatocyte a_K^i does not decrease during hypoosmotic stress was unexpected. One interpretation of this finding is that hepatocyte volumes do not change during osmotic stress. Instead, the cells may regulate their volumes by unknown mechanisms. To test this idea, hepatocyte steady-state relative volume was measured after cells equilibrated for 10 min in medium of various osmolalities (altered NaCl concentration). This was done in mouse hepatocytes in short-term (4 hr) primary monolayer culture to obviate problems of the diffusion delays and the anoxic interior of liver slices. The latter do not pertain to electrophysiologic measurements, because they were limited to cells on the surface of liver slices. V_m of mouse hepatocytes in primary monolayer (4 hr) is −35 mV (Wondergem & Castillo, 1987), which is in fair agreement with mouse hepatocyte *in situ* (Wondergem & Castillo, 1986). Also, Graf et al. (1987) have shown that membrane-relative ion selectivities in rat hepatocyte couplets compare favorably with those of the intact tissue. From this we conclude that membrane properties of mouse hepatocytes in short-term culture are similar to those *in situ*.

Mouse hepatocytes were exposed for 10 min at 37°C (Fig. 8, solid line) or at 4°C (Fig. 8, dashed line) to anisosmotic medium of altered NaCl concentration. At 37°C relative cell volume did not change in the range on the abscissa from 0.8–1.5. This suggests that the cells maintained their steady-state volume in this range of external osmotic stress. This did not occur when the experiment was repeated at 4°C. Now the cells behaved as expected osmometers where relative cell volume fit a predicted Boyle-Van't Hoff plot in the range of the abscissa from 0.8–1.5. Thus, the cells lost ability to regulate cell volume at 4°C. At 37°C cells lost their ability to maintain relative cell volume at the abscissa value beyond 1.5. Visual inspection of the cells by phase-contrast light microscopy showed bleb formation by the plasma membranes of hepatocytes at 37°C during this degree of hypoosmotic stress.

Discussion

Adaptive, cell volume regulation is an important physiologic problem for euryhaline fishes and vari-

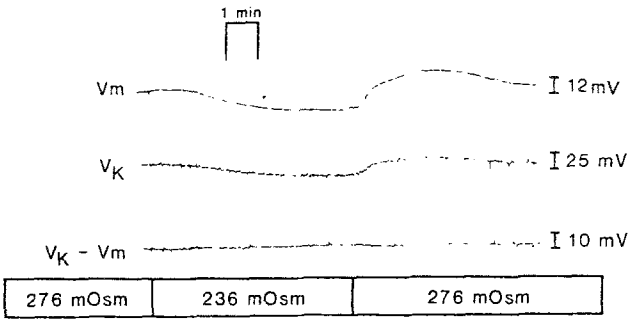

Fig. 7. Effect of hypoosmotic stress on intracellular voltages recorded with a double-barreled K-selective microelectrode. V_m shows voltages recorded with the open-tip or reference microelectrode. V_K shows voltages recorded with the liquid-exchanger K-selective microelectrode (Corning 477317). $V_K - V_m$ shows continuous recording of the differential voltages of the K-selective and reference microelectrodes. Downward movement indicates an increase in potential for all voltage traces. The calibration slope for this K-selective microelectrode was 57 mV/tenfold change in K^+ activity, and the selectivity for K^+ over Na^+ was 78. The reference microelectrode was filled with 0.5 N NaCl

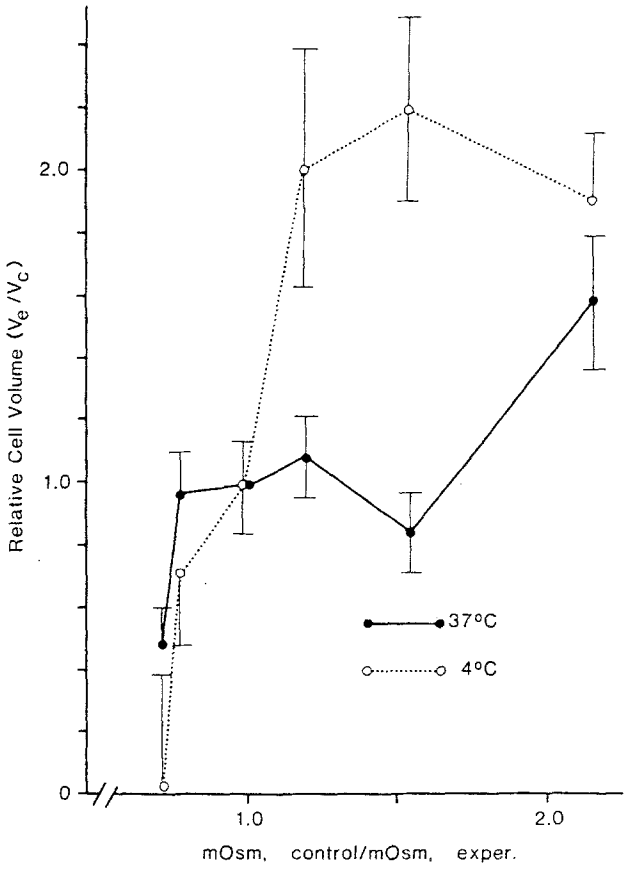

Fig. 8. Hepatocyte relative steady-state volume plotted as a function of external medium relative osmolality. Mouse hepatocytes in primary monolayer culture (4 hr) were exposed for 10 min at 37°C (solid line) or at 4°C (dotted line) to an isosmotic medium of altered NaCl concentration. Each point is the mean ± SE of six measurements

ous invertebrate, benthic organisms living in tidal pools or brackish water. However, for mammals and other vertebrates, *prima facie* evidence and need for cell volume regulation has been questioned recently, considering that neuro-renal function normally regulates extracellular osmolality to a set point (Spring & Ericson, 1982). Experimental, anisosmotic challenges to cells from these animals probably trigger adaptive mechanisms that respond primarily to hormonally induced changes in membrane transport and/or cell metabolism. This may be singularly important for the hepatocyte plasma membrane.

Our results show that mouse hepatocyte V_m behaves as an osmometer when liver slices are exposed to anisosmotic medium. The results also show that hepatocytes at 37°C maintain steady-state relative cell volume throughout the range of extracellular osmolalities that cause changes in V_m. Taken together, we conclude that changes in membrane ion conductance that account for variation of hepatocyte V_m with osmolality comprise part of the homeostatic mechanisms regulating cell volume. Volume regulation by hepatocytes may be important to the cells' ability to counteract intracellular osmotic imbalance that may occur during glycogenolysis or during membrane transport of organic solutes, such as alanine, glucose, and bile acids.

We also conclude that increases in hepatocyte V_m following hypoosmotic stress result from increases in hepatocyte membrane gK. Kregenow (1971), using duck erythrocytes, first reported that hypotonic stress increases membrane K^+ permeability, resulting in cell K^+ loss. Similar increases in K^+ efflux have been shown to occur in hepatocytes following hypotonic shock and during cell swelling associated with alanine transport (Bakker-Grunwald, 1983; Kristensen & Folke, 1984).

Increases in membrane gK and V_m also occur in the basolateral membrane of *Necturus* enterocytes following cell swelling by hypotonic medium (Lau, Hudson & Schultz, 1984). Dellasega and Grantham (1973) reported cell volume regulation by rabbit renal tubules in hypotonic media that was inhibited by cooling, KCN, and increasing the external K^+ concentration. Our results showing that cooling and high external K^+ inhibit increases in V_m with hypotonic stress are consistent with the findings of Dellasega and Grantham, and they suggest that hepatocyte V_m changes are associated with cell volume regulation.

The changes in hepatocyte V_m with osmotic stress were unexpected. We predicted that cell swelling would dilute intracellular K^+ activity, reduce the K^+ equilibrium potential, E_K, and decrease

V_m. Instead, hepatocyte V_m increased with hypoosmotic stress, and a^i_K did not change. Thus, a^i_K is regulated but V_m varies during osmotic imbalance. Cell K^+ is essential for protein synthesis (Lubin, 1967), therefore, stable a^i_K may be important for proper hepatic enzyme function and metabolic reactions.

Nevertheless, Alpini et al. (1986) reported a large, hepatocyte membrane H_2O permeability coefficient of 98.6×10^{-5} cm/sec. Consequently, it is surprising that our external hypoosmotic stress of ~86% resulted in no significant decrease in a^i_K. Clearly some mechanism functions to control hepatocyte a^i_K, and it is most likely in concert with the mechanism to regulate cell volume at 37°C. Volume regulation probably occurs within seconds in hepatocytes, considering the cells' high water permeability coefficient and relative small size. This is much faster than the period of solution exchange in the tissue bath plus the unknown diffusion delay within the liver slice. Therefore, rapid cell volume regulation probably attenuates any decrease in a^i_K resulting from slow changes in osmolality of solution bathing the liver cells.

We do not know the significance that changes in hepatocyte V_m have for cell K^+ homeostasis or for cell volume regulation. Increased membrane K^+ permeability and efflux have been associated with regulatory volume decrease in response to hypotonic stress in various cells (Kregenow, 1981; Davis & Finn, 1982; Grinstein, 1984), and this K efflux may be conductive (Lau et al., 1984). Although conductive K efflux from mouse hepatocytes does not change a^i_K significantly, the increase in V_m may provide electromotive force for transmembrane redistribution of osmotically active ions other than K^+. Recent findings demonstrate that in mouse and rat hepatocytes, transmembrane Cl^- distribution is passive with respect to V_m (Fitz & Scharschmidt, 1987*b*; Graf et al., 1987; Lyall, Croxton & Armstrong, 1987). A 20-mV increase in V_m from −40 to −60 mV would decrease intracellular Cl^- activity from 21 to 10 mM. Such redistribution of cell Cl^- could be electroneutral, in which case the 20-mV increase in V_m would reduce the intracellular ion osmolarity by ~22 mM. Regarding this, Van Rossum and Russo (1984), working with rat liver, "suggest that a cotransport of Na^+ and Cl^- forms an important part of the mechanism underlying ouabain-resistant water extrusion and, specifically, that this cotransport may take place across the membranes of the cytoplasmic vesicles."

Sucrose substitutions for the anisosmotic NaCl conditions indicated that the V_m changes did not result simply from altering the membrane gNa secondary to altered external Na^+ concentrations. We cannot explain why V_m decreased when external NaCl was lowered and osmolality kept constant with sucrose substitution, except to point out that this has been a consistent finding in our experiments. Henderson et al. (1986) have linked decreases in hepatocyte V_m with intracellular acidification secondary to inhibition of membrane Na-H exchange. The decreases in hepatocyte V_m by lowering external Na^+ reported here are consistent with this finding.

Variation of V_m with external osmolalities may have additional physiologic significance. Hepatic osmoreceptors associated with afferent nerve activity and with increases in plasma antidiuretic hormone have been inferred (Haberich, Aziz & Nowacki, 1965; Chwalbinska-Moneta, 1979; Baertschi, Massy & Kwon, 1985); however, morphological counterparts have not been identified. To what extent, if any, variation in hepatocyte V_m may serve osmoreceptor function and signal transduction for afferent nerve activity is unknown at this time.

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