Heat shock-induced protection and enhancement of Na\(^{+}\)-glucose cotransport by LLC-PK\(_1\) monolayers

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Sussman, Caroline R., and J. Larry Renfro. Heat shock-induced protection and enhancement of Na\(^{+}\)-glucose cotransport by LLC-PK\(_1\) monolayers. Am. J. Physiol. 273 (Renal Physiol. 42): F530–F537, 1997.—Monolayers of the porcine-derived renal epithelial cell line, LLC-PK\(_1\), were used to characterize the effects of heat stress on Na\(^{+}\)-glucose cotransport. Transepithelial current dependent on 5 mM glucose (I\(_{\text{Glc}}\)), phloridzin-sensitive current (I\(_{\text{phz}}\)), and total transepithelial current (I\(_{\text{total}}\)) were measured as indicators of Na\(^{+}\)-glucose cotransport. Severe heat shock (SHS; 45°C for 1 h, then 37°C for 30 min) decreased transepithelial electrical resistance (TER), I\(_{\text{Glc}}\), I\(_{\text{phz}}\), and I\(_{\text{total}}\). Mild heat shock (MHS; 42°C for 1 h, then 37°C for 12 h) induced accumulation of 72-kDa heat shock protein (HSP-72), decreased damage to TER from SHS, and prevented damage to I\(_{\text{Glc}}\), I\(_{\text{phz}}\), and I\(_{\text{total}}\). Kinetic analysis showed that SHS damaged and MHS protected total Na\(^{+}\)-glucose transport capacity (V\(_{\text{max}}\) of I\(_{\text{Glc}}\)). MHS alone increased TER (50%), I\(_{\text{Glc}}\) (20%), I\(_{\text{total}}\) (20%), and V\(_{\text{max}}\) of I\(_{\text{Glc}}\) (25%). On enhancement of the Na\(^{+}\) gradient by depletion of intracellular Na\(^{+}\), MHS increased I\(_{\text{Glc}}\) 50% and had no effect on transepithelial Na\(^{+}\)-dependent sulfate reabsorptive flux (50%) or on Na\(^{+}\)-dependent glucose cotransport. These effects of MHS were not reflected in effects on cell survival or luminal membrane surface area as indicated by lactate dehydrogenase or alkaline phosphatase release. In conclusion, HSP-72-inducing heat treatment both protected and enhanced Na\(^{+}\)-glucose cotransport independently of the luminal membrane Na\(^{+}\) gradient and selectively with respect to effects on TER, reabsorptive sulfate transport, cell survival, and luminal membrane surface area.

As with protective effects, many different stresses, e.g., ischemia (10) or exposure to bacterial endotoxin (20), induce HSPs.

We have previously shown that heat or zinc exposure induces HSP synthesis and accumulation in primary cultures of flounder renal proximal tubule. These treatments also prevent the decreases in transepithelial sulfate transport seen in uninduced tissues when exposed to more severe heat or chemical treatment (3, 28). This functional protection results from offsetting enhancement of sulfate transport rather than prevention of damage to transport (28). Similarly, renal multidrug resistance-like transport nearly doubles from the same heat shock treatment as that which stimulates and protects sulfate transport (30).

To determine whether mammalian renal proximal tubular transport was similarly affected, we assessed the effects of heat stress on Na\(^{+}\)-glucose and sulfate transport by LLC-PK\(_1\) monolayers. We observed phloridzin-inhibitable currents in the presence of glucose as has been reported for LLC-PK\(_1\) monolayers (22, 23). This is consistent with expression of a luminal Na\(^{+}\)-glucose cotransporter in LLC-PK\(_1\) cells (26), similar to that in mammalian renal proximal tubule associated with reabsorption of filtered glucose (1, 5). We report here transepithelial reabsorptive Na\(^{+}\)-dependent sulfate transport by LLC-PK\(_1\) monolayers consistent with luminal Na\(^{+}\)-sulfate cotransport, as in mammalian renal proximal tubule (19) associated with reabsorption of filtered sulfate. An HSP-72-inducing mild heat stress (MHS) protected Na\(^{+}\)-glucose cotransport capacity from damage by severe heat stress (SHS). Additionally, MHS selectively enhanced Na\(^{+}\)-glucose cotransport but had no effect on sulfate transport. These effects were not explained by changes in the plasma membrane Na\(^{+}\) gradient or by effects on cell survival or luminal membrane surface area.

MATERIALS AND METHODS

Cell culture. LLC-PK\(_1\) cells (15) were provided by Dr. Mary Taub (Depot. of Biochemistry, State University of New York, Buffalo, NY). Cells (screened for mycoplasma contamination) were grown to confluence on plastic before transfer to collagen gels. Cells were released from plastic by incubation in phosphate-buffered saline (PBS), containing (in mM) 140 NaCl, 2.7 KCl, 4.3 Na\(_2\)HPO\(_4\), 1.5 KH\(_2\)PO\(_4\), pH 7.3, plus 22 kU/ml trypsin (0.2%, Sigma, St. Louis, MO) and 1.8 mM EDTA for 5 min at 37°C. Growth medium was Dulbecco's modified Eagle's medium/Ham's F-12 (DMEM-F12) (50:50) (GIBCO, Grand Island, NY) without antibiotics, with 25 mM NaHCO\(_3\) and supplemented with 10% fetal bovine serum (HyClone, Logan, UT). Cells were maintained at 37°C in humidified 5% CO\(_2\)-95% air to give medium a pH of 7.4.
Previous work with winter flounder proximal tubule primary monolayer cultures showed that transepithelial transport was substantially improved by culture on contractible ("native" or undenatured) rat tail collagen. Na+-dependent glucose current was eightfold higher on contracted collagen compared with fixed, uncontracted collagen (7). In an effort to improve the rates of transepithelial transport, this same technique was applied here to LLC-PK1 cells. Cells (1·10⁵ cells/cm²) were plated on contractible collagen gels and maintained in the above medium plus 0.001% (wt/vol) each of insulin and hydrocortisone-21-phosphate (Sigma). Gels of rat tail collagen were prepared according to the method of Eldsland and Bard (9) modified by Dickman and Renfro (8). Collagen (0.5 mg × cm⁻²) was placed in culture wells, allowed to gel, rinsed twice with DMEM-F12, and equilibrated with growth medium plus hormones before use. Two days after plating, cells formed confluent monolayers. At that time, collagen gels were released from culture wells allowing monolayers to contract the floating gels.

Scanning electron microscopy. Monolayers were fixed in cacodylate-buffered glutaraldehyde-paraformaldehyde (1.5% each, pH 7.3) at room temperature for 4 h, postfixed with osmium tetroxide, and dehydrated through an ethanol series. Fixation and dehydration steps were carried out as above with the transition fluid (Polaron, E3000), sputter coated with gold (Polaron, E5100), and examined on a Coates and Welter field-emission scanning electron microscope (model HPSS50).

Heat shock treatment. After 15–20 days on collagen, tissues were heat shocked by partial submersion of culture dishes in a temperature controlled (± 0.1°C) water bath (Lauda RM6). Tissues were given either SHS (45°C for 1 h, then 37°C for measurements) or MHS (42°C for 3 h, then 37°C for 12 h) before placement in Ussing chambers. Tissues exposed to both mild and severe heat shock (MHS + SHS) received SHS instead of the last hour of recovery at 37°C after MHS. To provide uniform timing, tissues were returned to 37°C for 0.5 h before sample collection for enzyme assays (Ussing chamber setup required 0.5 h). The highest temperature at which no acute damage to transepithelial electrical resistance (TER) or transepithelial current was observed after a 1-h exposure and the lowest at which accumulation of HSP-72 was observed after a 2-h exposure indicated tissue damage, i.e., 70% decrease in TER and 50% decrease in transepithelial current, occurred after incubation of control tissues at 45°C for 1 h.

Gel electrophoresis and Western blots. Cells were removed from collagen gels by incubation with EDTA (1.8 mM in PBS) at 37°C for 15 min, EDTA plus trypsin (as above) at 37°C for 10 min, and vigorous rinsing. Unattached cells were suspended in and washed once with PBS (4°C) and then lysed with Kaman buffer [62.5 mM trizma base, pH 6.8, 2.3% (wt/vol) sodium dodecyl sulfate (SDS), 5% (vol/vol) β-mercaptoethanol, 10% (vol/vol) glycerol].

Proteins were separated by one-dimensional SDS-10% polyacrylamide gel electrophoresis, as described by Laemmli (17). Gels were silver stained, or proteins were electroblotted to nitrocellulose and probed with monoclonal antibody SPA 820 against both constitutively expressed and inducible human 70-kDa heat shock proteins (Hsc-73 and HSP-72, respectively) or SPA 810, which is specific for HSP-72 (Stressgen, Victoria, BC, Canada). Reactiv proteins were visualized with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (HyClone) and 3′-3-diaminobenzidine.

Transport and electrical characteristics. Confluent cultures supported by 150-μm nylon mesh were placed in Ussing chambers. Measurements were conducted in HCO₃⁻-buffered saline containing (in mM) 1.1 CaCl₂, 4.2 KCl, 0.3 MgCl₂, 0.4 MgSO₄, 120 NaCl, 25 NaHCO₃, 0.4 NaH₂PO₄, 0.5 Na₂HPO₄, 1.0 l-glutamine, and 5 glucose. Chambers were continuously gassed with humidified 5% CO₂–95% O₂ and maintained at 37°C, so that buffer was pH 7.5. For measurements of Na⁺-dependent transport, luminal solution only was buffer of identical composition, except that NaCl and NaHCO₃ were replaced isosmotically with trizma base, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, N-methyl-d-glucamine (NMDG), and HCl to pH 7.5 (Na⁺ substitution buffer). Buffer of identical composition, except containing Na⁺ instead of NMDG, was then added for subsequent measurements (Na⁺ replacement buffer).

Electrical characteristics of tissues were monitored with two automatic dual voltage clamps (World Precision Instruments, Waltham, MA) interfaced with a data acquisition system (MacLab). Ag/AgCl electrodes were connected to the peritubular and luminal compartments with 3 M KCl-2% agar bridges. TER was calculated from the change in potential difference (PD) that resulted from a 10-μA current pulse (40 ms). Background resistance was calculated for each chamber as above after cells and collagen were removed from the chamber aperture by scraping. Preliminary tests showed that identical background resistances were obtained by adding 150 mM EDTA to each hemichamber to disrupt tight junctions. Transepithelial current was calculated by dividing PD by TER. Glucose-dependent current (IGLU) was calculated as the difference between the currents observed ± 5 mM glucose. Kinetics of IGLU were determined from Eadie–Hofstee plots obtained by the sequential addition of glucose to both peritubular and luminal hemichambers. Phloridzin-sensitive current (Iphz) was determined in the presence of 5 mM glucose by the addition of 0.2 mM phloridzin to the luminal side. This concentration of phloridzin caused maximal inhibition of current and did not affect current when added to the peritubular side only.

Unidirectional sulfate transport by 15- to 20-day-old tissues was measured by addition of 35SO₄²⁻ (ICN, Costa Mesa, CA) to either the peritubular or luminal hemichamber. During measurement of net sulfate fluxes, tissues were short circuit, i.e., tissues had identical peritubular and luminal buffers and were voltage clamped at zero transepithelial PD. Duplicate 50-μl aliquots from the opposite hemichamber were sampled at 30-min intervals with volume replacement for 2–3 h. At the end of the 3-h run, the Na⁺-dependent component of reabsorptive sulfate flux, tissues were not voltage clamped. 35SO₄²⁻ was added to the luminal side only, and duplicate 50-μl aliquots were sampled from the peritubular hemichamber every 10 min with volume replacement. Samples were collected for 30 min before and 30 min after addition of luminal Na⁺. Na⁺-dependent sulfate flux was calculated as the difference between the sulfate flux before and 10 min after addition of Na⁺. Glucose-dependent PD was 0.3 ± 0.01 mV (lumen negative) with Na⁺ substitution buffer (low Na⁺ concentration ([Na⁺]) on the luminal side. This PD rose to −1.1 ± 0.09 mV when Na⁺ was replaced (Na⁺ replacement buffer) on the luminal side. This effect was consistent with prolonged maintenance of a lower luminal Na⁺ concentration while Na⁺ substitution buffer was present.

Enzyme assays. Aliquots of medium were collected from 15- to 20-day-old tissues to quantify enzyme release since the last feeding (48 h). Cells were then lysed by addition of 9% Triton X-100 in PBS to culture medium (1:10, vol/vol) and incubation at 37°C for 45 min. PBS-Triton X-100 was also added to aliquots collected before cell lysis. Lactate dehydrogenase (LDH) assays were conducted using a kit (CytoTox; Promega, Madison, WI). Alkaline phosphatase (AP) was assayed as previously described (31).
RESULTS

Characteristics of LLC-PK1 monolayers on contractile collagen. Monolayers were confluent 2 days after plating inaccurate and contracted collagen gels for at least 20 days after plating (Table 1). During this time, cells changed shape from squamous to cuboidal and developed luminal cilia and dense luminal microvilli (Fig. 1). TER decreased, and currents increased with time after plating but were stable between 15 and 20 days after plating, at which time all subsequent experiments were done. Similar changes in characteristics of LLC-PK1 monolayers with time after plating have been observed previously (21, 25).

Table 1. Electrical characteristics of LLC-PK1 monolayers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Age, days after plating</th>
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<tbody>
<tr>
<td>Diameter, mm</td>
<td>4–5 10–11 15–16 18–20</td>
</tr>
<tr>
<td>TER, Ω·cm²</td>
<td>30.6 ± 0.4 26.9 ± 0.6 20.9 ± 0.7 17.8 ± 0.9</td>
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<tr>
<td>I_total, µA/cm²</td>
<td>10.1 ± 0.6 21.0 ± 1.0 24.3 ± 1.4 23.3 ± 1.2</td>
</tr>
<tr>
<td>I_Glc, µA/cm²</td>
<td>ND 18.8 ± 1.2 21.5 ± 1.3 22.3 ± 1.0</td>
</tr>
<tr>
<td>I_phe, µA/cm²</td>
<td>8.0 ± 0.7 13.8 ± 1.2 15.4 ± 1.4 14.0 ± 1.1</td>
</tr>
<tr>
<td>V_max, µA/cm²</td>
<td>ND 16.9 ± 0.8 21.8 ± 1.1 23.8 ± 0.8</td>
</tr>
<tr>
<td>K_m (mM)</td>
<td>ND 0.21 ± 0.02 0.3 ± 0.02 0.35 ± 0.03</td>
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</table>

Values are means ± SE. TER, transepithelial electrical resistance; I_total, total transepithelial current with 5 mM glucose; I_Glc, transepithelial current dependent on 5 mM glucose; I_phe, transepithelial current sensitive to 0.2 mM phlorizin; V_max, maximum velocity of glucose-dependent current; K_m, glucose concentration yielding 0.5 V_max. Kinetics were determined by Eadie-Hofstee plot. ND, no data. *†§Significantly different from corresponding variable in 4- to 5-, 10- to 11-, or 15- to 16-day-old cultures, respectively (P < 0.05, n = 8–33).

I_Glc comprised 92% of total current in 15- to 20-day-old cultures (Table 1). This current represented Na⁺-glucose cotransport and was not attributable to a metabolic effect of glucose, a previously reported observation for this cell line (22). Luminal phlorizin, an inhibitor of luminal Na⁺-glucose cotransport (1), inhibited 67% of the I_Glc. Phlorizin had no effect when added at the peritubular side (data not shown). Similarly, peritubular glucose had no effect on current (data not shown). Addition of 5 mM α-methylglucoside (AMG), a nonmetabolizable glucose analog (25), elicited 83% of I_Glc. The increase in lumen-negative PD on glucose addition was dependent on the presence of luminal Na⁺; the glucose-dependent PD change when luminal solution only was replaced with Na⁺ substitution buffer in which NMDG-HCl replaced NaCl was 0.3 ± 0.01 vs. 1.1 ± 0.09 mV with Na⁺ replacement buffer.

Kinetic data were described by a straight line on Eadie-Hofstee plots (Fig. 2). The similarity between Na⁺-glucose transport capacity (V_max) and I_Glc indicated that 5 mM glucose (the concentration used to determine I_Glc) elicited maximum glucose transport (Table 1). Changes in Michaelis constant (K_m) with time showed that affinity of the transporter for glucose or Na⁺ decreased slightly as V_max increased. Current induction by glucose and AMG, sensitivity to luminal phlorizin, and K_m ~0.3 mM (Tables 1 and 2) are consistent with previous observations in LLC-PK1 cells (22, 23, 25).

Accumulation of HSP-72 following MHS. Exposure to 42°C for 3 h induced accumulation of HSP-72 4–48 h after return to 37°C (Fig. 3). After 48 h, a decline toward control was evident. Changes in accumulation of HSP-72 occurred in the absence of changes in...
accumulation of its constitutive form, Hsc-73. Because tissues were not gassed, there was a rise in medium pH during heat shock. To test whether this elevated pH could induce HSP-72, we examined its accumulation after incubation in a water bath for 3 h at 37°C without gassing. No accumulation of HSP-72 was observed.

Effects of MHS and/or SHS on TER and glucose transport. LLC-PK1 monolayers were placed in Ussing chambers immediately after incubation at 45°C for 1 h (SHS) or 12 h after incubation at 42°C for 3 h (MHS) for measurement of TER and currents. I_Glc decreased 70% after SHS and increased 20% after MHS (Fig. 4). The effect of SHS was eliminated by previous MHS; there was no difference between control I_Glc and that after MHS + SHS. SHS following MHS did, however, decrease I_Glc 20% compared with I_Glc with MHS alone. Protection was therefore achieved by a combination of prevention of damage (20% vs. 70%) and offsetting of damage by previous enhancement (20%). The effect of MHS on I_Glc was greater when the plasma membrane Na^+ gradient was enhanced; under these conditions, MHS increased I_Glc almost 50% (Fig. 8).

Like I_Glc, TER decreased 70% in response to SHS. MHS increased TER 50% compared with controls and decreased the effect of SHS; MHS + SHS decreased TER only 20% relative to control and 50% relative to MHS (Fig. 5). In contrast to protection of I_Glc, the partial protection of TER observed was achieved primarily by MHS-induced enhancement (50%) and not by prevention of damage (50 vs. 70%).

Effects of heat shock on phloridzin-sensitive current (I_phz), total transepithelial current (I_total), and V_max of I_Glc were attributable to effects on I_Glc (Table 2). I_phz decreased 50% following SHS but was not changed by MHS + SHS. I_total decreased 70% after SHS and increased 20% after MHS. When preceded by MHS, SHS had no effect relative to control and decreased I_total 20% relative to MHS. V_max decreased 60% following SHS, increased 25% following MHS, and was not affected by MHS + SHS relative to control. MHS +

### Table 2. Effects of heat shock on I_total, I_phz, and kinetics of glucose-dependent current

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SHS</th>
<th>MHS</th>
<th>MHS + SHS</th>
</tr>
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<tbody>
<tr>
<td>I_total, µA/cm²</td>
<td>19.8 ± 1.3</td>
<td>5.5 ± 1.0*</td>
<td>23.5 ± 1.1†</td>
<td>18.6 ± 1.4†</td>
</tr>
<tr>
<td>I_phz, µA/cm²</td>
<td>12.5 ± 1.4</td>
<td>6.7 ± 1.2*</td>
<td>13.8 ± 0.9†</td>
<td>14.9 ± 1.5†</td>
</tr>
<tr>
<td>V_max, µA/cm²</td>
<td>20.7 ± 1.7</td>
<td>8.1 ± 1.2*</td>
<td>25.4 ± 1.4†</td>
<td>21.8 ± 1.9†</td>
</tr>
<tr>
<td>K_m, mM</td>
<td>0.2 ± 0.02</td>
<td>0.3 ± 0.06</td>
<td>0.3 ± 0.03*</td>
<td>0.4 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *†Significantly different from corresponding control, severe heat stress (SHS), and mild heat stress (MHS) tissues, respectively (P < 0.05, n = 8–12). Similar results were observed for all variables in 34–46 additional control and MHS tissues.
SHS decreased $V_{\text{max}}$ 10% relative to MHS. $K_m$ increased following both MHS and MHS + SHS.

MHS and sulfate transport. Unidirectional sulfate fluxes were stable between 1 and 2 h after addition of $\text{SO}_4^{2-}$; therefore, sulfate fluxes measured at 1, 1.5, and 2 h were averaged to obtain values for steady-state unidirectional flux. Under short-circuit conditions, net reabsorption occurred in both control and MHS tissues, i.e., luminal-to-peritubular (reabsorptive) flux was greater than peritubular-to-luminal (secretory) flux (Fig. 6). Controls had secretory and reabsorptive fluxes of $2.9 \pm 0.3$ and $4.0 \pm 0.4$ nmol·cm$^{-2}$·h$^{-1}$, respectively. Thus tissues performed active transepithelial reabsorptive sulfate transport. MHS decreased secretory and reabsorptive fluxes to $2.0 \pm 0.2$ and $3.3 \pm 0.3$ nmol·cm$^{-2}$·h$^{-1}$, respectively, i.e., by similar amounts relative to controls and therefore did not affect net sulfate flux.

Na$^+$-independent reabsorptive sulfate flux in both control and MHS tissues reached steady state within 10 min (Fig. 7). Sulfate flux was greatest 10 min after replacement of Na$^+$ and declined steadily thereafter. Reabsorptive sulfate fluxes before and after addition of Na$^+$ were lower with MHS than control.

Na$^+$-dependent sulfate flux was calculated by subtracting the steady-state sulfate flux in the absence of Na$^+$ (average of 10-, 20-, and 30-min samples) from the sulfate flux measured 10 min after replacement of Na$^+$ (Figs. 7 and 8). Na$^+$-dependent sulfate flux was not affected by MHS. Controls had $4.8 \pm 0.5$ vs. $4.6 \pm 0.4$ nmol·cm$^{-2}$·h$^{-1}$ in MHS tissues. In contrast to the lack of effect on sulfate flux, MHS increased $I_{\text{Glc}}$ almost 50% in the same tissues from $14.0 \pm 0.8$ to $20.3 \pm 1.9$ µA/cm$^2$ (Fig. 8).

Effect of MHS and/or SHS on enzyme release. Samples were collected for enzyme assays from heat-shocked tissues either 30 min (SHS) or 12 h (MHS) after return to 37°C. This timing is the same as that used for flux and electrical measurements. Relative to control, SHS caused release of 30% more LDH (Fig. 9). MHS increased LDH release to a lesser extent (20%). LDH release after MHS + SHS was the same as that after SHS alone. If LDH release resulted from cell death, then SHS caused more cell death than MHS, and MHS did not prevent cell death following subsequent SHS.

All heat shock treatments increased the percent of total AP released by approximately the same amount (Table 3). Because heat shock could affect total AP levels, AP release was also expressed as a fraction of total LDH (as an indicator of cell number). This calculation yielded the same results. Additionally, the ratio of

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**Fig. 6.** Effect of MHS on transepithelial sulfate flux. Control or MHS monolayers were placed in Ussing chambers for measurement of transepithelial sulfate flux under short-circuited conditions. Hatched bars, average net flux. Data are means ± SE. *Significantly different from control peritubular-to-luminal flux ($P < 0.05$, n = 13).

**Fig. 7.** Effect of MHS on reabsorptive sulfate flux. Control or MHS tissues were placed in Ussing chambers with low luminal Na$^+$ (Na$^+$ substitution buffer). After 30 min, luminal Na$^+$ was replaced (Na$^+$ replacement buffer). Solid line, control; dashed line, MHS. Data are means ± SE. *Significantly different from corresponding control data points ($P < 0.05$, n = 16-20).

**Fig. 8.** Effect of MHS on Na$^+$-dependent sulfate flux and glucose-dependent transport ($I_{\text{Glc}}$). Na$^+$-dependent reabsorptive sulfate transport was calculated by subtracting steady-state flux in the absence of Na$^+$ from that measured 10 min after Na$^+$ replacement. $I_{\text{Glc}}$ was measured immediately following addition of Na$^+$ to the luminal hemichamber. Data are means ± SE. *Significantly different from corresponding control ($P < 0.05$). Open bars, sulfate flux (n = 16-20); solid bars, $I_{\text{Glc}}$ (n = 6-7).
Table 3. Effects of MHS and/or SHS on AP and LDH release

<table>
<thead>
<tr>
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<th>SHS</th>
<th>MHS</th>
<th>MHS + SHS</th>
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<tr>
<td>AP released, % of total</td>
<td>1.23 ± 0.02*</td>
<td>1.16 ± 0.04*</td>
<td>1.20 ± 0.02*</td>
</tr>
<tr>
<td>AP released/total LDH</td>
<td>1.25 ± 0.03*</td>
<td>1.22 ± 0.01*</td>
<td>1.23 ± 0.04*</td>
</tr>
<tr>
<td>AP released/LDH released</td>
<td>0.96 ± 0.05</td>
<td>1.03 ± 0.02</td>
<td>0.95 ± 0.07</td>
</tr>
<tr>
<td>Total AP</td>
<td>1.03 ± 0.05</td>
<td>1.07 ± 0.01</td>
<td>1.07 ± 0.03</td>
</tr>
<tr>
<td>Total LDH</td>
<td>1.01 ± 0.02</td>
<td>1.02 ± 0.03</td>
<td>1.04 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 groups of 4 tissues assayed concurrently. *§†Significantly different from control, SHS, and MHS, respectively (P < 0.05).

AP released to LDH released was the same as control for all treatments. If AP release resulted from loss of microvilli, then there was no selective damage to or protection of luminal microvilli by heat shock, and loss of microvilli was accounted for by cell death in all treatments. That total AP and LDH extracted were the same in all cases confirmed that results were not biased by differences in enzyme extraction efficiency.

**DISCUSSION**

These data demonstrate the protection and enhancement of a vital renal function, reabsorptive Na⁺-glucose cotransport, by mild heat stress. Retention of enhancement on manipulation of the plasma membrane Na⁺ gradient by depletion of intracellular Na⁺ indicated that this effect was not due to an altered plasma membrane Na⁺ gradient. Similarly, kinetic analysis indicated that MHS increased total glucose transport capacity. The lack of enhancement of reabsorptive sulfate fluxes indicated that enhancement was not due to an all-encompassing change in tissue condition. This is also consistent with a lack of effect on the plasma membrane Na⁺ gradient since the luminal steps in both sulfate and glucose transport processes are Na⁺ dependent. No cause and effect relationship between HSP induction and Na⁺-glucose cotransport can be inferred from the present data. Protective and enhancing effects were associated with increased levels of HSP-72 but not with LDH nor AP released by monolayers, indicating that effects of MHS were independent of effects on cell survival or luminal membrane surface area.

Protective effects of MHS. The protective effects shown in the present study may be related to the observation that, after ischemia and transplantation, kidneys with heat stress-induced HSP-72 have improved function and their recipients have higher survival rates (27). Three indicators of Na⁺-glucose cotransport (I_{Glc}, I_{total}, and I_{glu}) showed that it was protected by MHS (Fig. 4, Table 2). In addition, protection of V_{max} of glucose-dependent current (Table 2) demonstrated that total Na⁺-glucose cotransport capacity was protected. These effects were primarily due to prevention of damage following SHS. Similarly, previous MHS reduced the decrease in TER following SHS; however, unlike the effect on I_{Glc}, this effect was primarily due to an offsetting increase in TER following MHS and not to prevention of damage (Fig. 5).

We have previously observed protection of renal proximal tubule epithelial transport utilizing primary monolayers of flounder renal tubule. Decreases in sulfate secretory transport (active in flounder kidney) with normally injurious thermal or chemical treatment are prevented by previous heat or zinc exposure (3, 28). This functional protection results from enhancement of transport rather than prevention of damage (28). Thus certain transport processes in both mammalian and teleost renal tubules are protected by mild stress; however, the mechanism appears to be different.

Additional examples of functional protection by HSP-72-inducing heat stress have been reported. Endotoxin exposure and heat shock increase resistance of cardiac activity to endotoxemic myocardial depression and postschismic dysfunction (6, 20). In the Madin-Darby canine kidney cell line, an HSP-72-inducing heat stress protects TER from decreases following further heat stress (24).

Additional effects of MHS. In addition to protecting Na⁺-glucose cotransport, MHS increased I_{Glc} (Fig. 4) and V_{max} and K_m of glucose-dependent current (Table 2). Thus enhancement resulted from an increase in the capacity of the epithelium for glucose transport. The effect of MHS on I_{Glc} was retained, despite manipulation of the Na⁺ gradient (Fig. 8), indicating that this effect was not due to alteration of the plasma membrane Na⁺ gradient. The reasons for enhancement of transport are unknown but may reflect an increase in the number, activity, or type of transporter(s). The decreased affinity for glucose could reflect partial unfolding of the transporter due to heat-induced denaturation.

Enhancement of membrane transport by HSP-inducing stress has been observed in several cell types for several substrates. Both the expression and function of a multidrug resistance transporter, p-glycoprotein, are upregulated in response to several forms of stress (4). Heat stress increases transport of glutathi-
Net sulfate reabsorption occurred in both control and MHS tissues under short-circuit conditions (Fig. 6). Neither net sulfate flux nor the Na\(^+\)-dependent component of reabsorptive sulfate flux were affected by MHS; however, this treatment decreased both unidirectional fluxes as well as the separately measured reabsorptive sulfate fluxes before and after Na\(^+\) addition (Figs. 6 and 7). These decreases may reflect the passive component of sulfate flux as is consistent with the increased TER caused by MHS. That net and the Na\(^+\)-dependent component of sulfate transport were not affected by MHS (Figs. 6 and 8), unlike the fluxes containing the passive component of flux, is consistent with this hypothesis. The data strongly suggest specific enhancement by MHS of Na\(^+\)-glucose cotransport at the level of the transporter itself. This is indicated by the effects of MHS on \(V_{\text{max}}\) of glucose-dependent current; retention of the effect despite manipulation of the Na\(^+\) gradient, and the lack of effect on reabsorptive sulfate transport. It is likely that SGLT1, an Na\(^+\)-dependent glucose transporter found in LLC-PK\(_1\) cells (21, 26), is the transporter responsible for glucose transport observed here. Phloridzin sensitivity and current induction by AMG are characteristics of activity of this transporter (14), which is also found in mammalian renal epithelial cells in vivo (5). Therefore, data suggest that the activity of this transporter can be modulated by heat shock.

Efficient reabsorption of filtered glucose by the renal proximal tubule is critical for survival. It is possible that enhancement of reabsorptive glucose transport maintains this efficiency in times of stress. TER was not protected to the same extent as glucose transport (Figs. 4 and 5). To maintain glucose reabsorptive efficiency despite decreased TER and, hence, increased leakiness, active transport must increase. Alternatively increased transport may result from modifications in the transporter associated with a state of increased tolerance of more severe stress.

The mechanistic basis of the effect of MHS on TER (Fig. 5) remains unresolved but most likely reflects an effect on the paracellular pathway. This could be caused by changes in tight junctions or perhaps cell swelling and partial occlusion of the paracellular pathways.

Effects of heat shock on HSP-72 and enzyme release. MHS caused accumulation of HSP-72 above control levels. This indicated that the heat shock response, of which HSP-72 induction is part, was activated. Our data do not provide evidence that the effects of MHS are dependent on HSP-72; however, HSP-72 performs many functions that could confer the protective effects observed. Through its chaperoning function (2), HSP-72 could bind and reduce heat-induced damage to important structural proteins or transport proteins.

Assays of LDH and AP indicated that protective and enhancing effects of MHS on Na\(^+\)-glucose cotransport could not be explained by effects on cell death or luminal microvilli. MHS reduced the LDH release due to SHS by one-half; when LDH release due to MHS was subtracted from that due to MHS + SHS, it is seen that half as many cells were killed by SHS following MHS than by SHS alone (Fig. 9). This could be due to increased thermotolerance or to noncumulative effects, i.e., sensitivity of the same population of cells to killing by SHS as by MHS. Regardless, it is worth noting that the decrease in Na\(^+\)-glucose cotransport (indicated by \(I_{\text{Glc}}\) \(I_{\text{phz}}\) \(I_{\text{total}}\) and \(V_{\text{max}}\)) following MHS + SHS, compared with MHS alone, was much less than would be predicted from the amount of cell death, i.e., half as much as the decrease following SHS alone (Fig. 4, Table 2). This was not true for TER, which decreased about the same amount due to MHS + SHS (relative to MHS) as due to SHS alone (relative to control) (Fig. 5). Protection of TER can, therefore, be explained by cell survival, but protection of \(I_{\text{Glc}}\) cannot. All heat shock treatments caused equivalent loss of AP, and AP released can be accounted for by cell death (ratios to LDH released were the same as control for all treatments) (Table 3).

Several characteristics of the monolayers used here were superior to those previously reported for LLC-PK\(_1\) cells. This is apparently the first demonstration of active sulfate reabsorption by any mammalian kidney-derived cell line. TER and currents were more similar to those for in vivo mammalian proximal tubule than those previously reported (22, 23). It is possible that these improvements resulted from the use of a contractile collagen substratum. The behavior of LLC-PK\(_1\) epithelium on contractile collagen substratum has not previously been characterized, and similar substrata have improved characteristics of other cultured epithelia (11, 32).

In summary, the present data show that an HSP-72-inducing heat shock can produce relatively specific protection and enhancement of Na\(^+\)-glucose cotransport, the first step in renal reabsorption of filtered glucose. The importance of protecting and thus maintaining normal tissue function during conditions of stress is clear. Specific protection and enhancement of glucose transport may serve to maintain function, despite cell or tissue injury. Cells may therefore act strategically during stress to protect certain critical functions and leave others unprotected.

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