Effect of heat stress on rabbit esophageal epithelium

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Tobey, Nelia A., Dipali Sikka, Esteban Marten, Canan Caymaz-Bor, S. Seraj Hosseini, and Roy C. Orlando. Effect of heat stress on rabbit esophageal epithelium. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1322–G1330, 1999.—Hot beverages expose the esophageal epithelium to temperatures as high as 58°C. To study the impact of such temperatures, rabbit esophageal epithelium was exposed to luminal heat or both luminal and serosal heat while mounted in Ussing chambers. Luminal heat, mimicking exposure to hot beverages, reduced potential difference (PD) and resistance (R) when applied at ≥49°C and reduced short-circuit current (Isc) at ≥60°C. At ≥60°C, subepithelial blisters developed. Higher temperatures reduced R only moderately and reversibly. In contrast, the Isc declined sharply and irreversibly once threshold was reached. Luminal and serosal heat also reduced PD, Isc, and R, although the threshold for reduction in Isc was now similar to that for R. Additionally, luminal and serosal heat reduced Isc more than R for any given temperature and resulted in blisters at lower temperatures (50°C) than luminal heat alone. The heat-induced decline in Isc was attributed in part to inactivation of Na-K-ATPase activity, although other transport systems could have been equally affected, and the decline in R to an increase in paracellular permeability. The latter effect on R also contributed to an increase in tissue sensitivity to luminal acid damage. Consumption of hot beverages exposes the esophagus to temperatures that can negatively impact epithelial structure and function. Impaired barrier function by heat increases the risk of esophageal damage by subsequent contact with (refluxed) gastric acid. These findings help explain in part the association between esophageal disease and consumption of hot beverages.

hydrochloric acid; potential difference; resistance; short-circuit current

HUMANS ARE UNIQUE in their preference for eating food and drinking beverages at high temperatures. Indeed in restaurants within the United States, hot beverages are served at temperatures up to 80°C (176°F; investigator survey), and yerba mate is eaten in Argentina at similar temperatures (5). In England, three separate studies indicated that healthy subjects prefer their hot coffee or tea at temperatures as high as 65°C, 68°C, and 76°C, with two reports noting that temperature preferences did not differ among males and females (2, 6, 14). Other studies indicate that 22% of English, 43% of Dutch, and 14% of Swedish subjects have temperature preferences for food and beverages that exceed 60°C. Notably, however, when hot beverages are consumed, the oral cavity modulates these temperatures so that the esophagus in actuality experiences temperatures well below those of the ingested liquid. For example, De Jong and colleagues (3) showed that sipping a hot beverage at temperatures ranging from 55 to 65°C resulted in increases in distal esophageal (luminal) temperatures averaging from 5 to 12°C above body temperature depending on bolus size (minimum 5 ml to a maximum of 20 ml), with the maximum intraluminal temperature observed for any individual being 53°C. Because this study demonstrated a linear relationship between temperature of the ingested bolus and temperature in the lumen in the distal esophagus, ingestion of hot beverages up to 80°C could be extrapolated from these data to elevate distal esophageal luminal temperature and so expose the esophageal epithelium to temperatures as high as 58°C (range 48–58°C).

Because it is known that the tertiary structure of proteins is destroyed at 43°C and cultured cells are destroyed at temperatures of 47°C (8, 14), it has long been recognized that ingestion of such hot substances has the potential to damage the tissues in its path. Indeed, consumption of hot beverages has been previously linked with a number of diseases of the upper gastrointestinal tract, including reflux esophagitis, gastritis, gastric ulcer, duodenal ulcer, and esophageal carcinoma (4, 14, 18, 19). Particularly notable was a study by Pearson and McCloy (14) in which they determined the temperature preference for consumption of hot beverages among patients with various disorders of the upper gastrointestinal tract and compared them to healthy controls. The results indicated that subjects with acid-peptic disease of esophagus, stomach, and duodenum preferred their beverages at significantly hotter temperatures than did the healthy subjects, with median temperature preference of those with esophageal disease (63.5°C) being the hottest for any group. Moreover this difference could not be accounted for by any difference in age, sex, smoking history, or choice of beverage (i.e., coffee or tea) among the groups.

Despite these intriguing observations, little is known about the effects of heat on the esophageal epithelium. For this reason, we exposed the esophageal epithelium of the rabbit to heat in the Ussing chamber and did so in two different ways. One method was to expose it to heat from the luminal side only to mimic the exposure as it occurs in vivo in humans consuming hot beverages. The second method was to expose it to heat from both luminal and serosal sides, a means of heat damping tissues to study the impact of specific temperatures.

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MATERIALS AND METHODS

New Zealand White male rabbits weighing between 8 and 9 pounds were killed by administering intravenously an overdose of pentobarbital (60 mg/ml). The esophagus was excised, opened, and stripped of its muscle layers in a paraffin tray containing ice-cold oxygenated Ringer solution so that a sheet of tissue was obtained consisting of stratified squamous epithelium and a small amount of underlying connective tissue. From this tissue, four sections were cut and mounted as flat sheets between Lucite half-chambers with an aperture of 1.13 cm² for measurements of potential difference (PD), short-circuit current (Isc), and calculation of electrical resistance (R). All tissues were bathed with Ringer solution (composition in mM): 140 NaCl, 119.8 Cl⁻, 5.2 K⁺, 25 HCO₃⁻, 1.2 Ca²⁺, 1.2 Mg²⁺, 2.4 HPO₄²⁻, and 0.4 H₂PO₄⁻, and gassed with 95% O₂-5% CO₂. Mucosal and serosal solutions were connected to calomel and Ag/AgCl electrodes with Ringer agar bridges for measurements of PD and automatic short-circuiting except for 5–10 s when the open circuit PD was connected to calomel and Ag/AgCl electrodes with Ringer solution at two consecutive 45-min intervals. Mannitol fluxes were determined using counts obtained from a liquid scintillation counter. The mean value for the two 45-min fluxes was reported as the value for the tissue.

RESULTS

To mimic esophageal exposure to hot beverage ingestion, rabbit esophageal epithelium mounted in Ussing chambers was initially exposed to heat by warming the luminal, but not the serosal, bathing solution; rapid changes in temperature were achieved by switching the tubing of the original bath at 37°C to one from an adjacent Ussing chamber preheated to the desired higher temperature. Therefore, by disconnecting and reconnecting the bathing solution tubing, the luminal bath temperature could be rapidly raised and then lowered back to 37°C.

For the experiments, the effect of heat stress on the ability of the tissue to resist luminal acid injury was assessed in the Ussing chamber. This was done by initially pairing three tissues by (within 25%; note 4–5 tissues can be obtained from the same rabbit esophagus) and then heating two of the three to a fixed temperature. After a fixed time period, the unheated tissue (unheated, acid-treated control) and one of the pair of heat-clamped tissues were acidified by the luminal addition of 60 mM HCl (pH 1.7) for 1 h. The other heated tissue was used as a nonacidified, heat-clamped control. Equimolar choline chloride was added to the serosal bath during HCl exposure to reduce osmolar effects and to balance the chloride concentration. Tissue damage was then assessed by monitoring subsequent changes in R and by morphology. Tissues evaluated morphologically were fixed for light microscopy in the chamber using 3% glutaraldehyde and later stained using hematoxylin and eosin. Morphological injury was assessed by an observer who had no knowledge of treatment groups using the scoring system: 0, normal; 1, intracellular or intercellular edema; 2, patchy necrosis; 3, diffuse necrosis; and 4, transmucosal necrosis (ulceration).

Mannitol flux measurements. Mannitol fluxes were measured in some experiments by the addition of both “cold” mannitol to Ringer solution to bring its concentration to 10 mM and “hot” [¹⁴C]mannitol (ICN, Irvine, CA; 10 µCi) to the luminal bath. After taking the “hot” side sample, 45 min were allowed for equilibration before sampling the serosal bathing solution at two consecutive 45-min intervals. Mannitol fluxes were determined using counts obtained from a liquid scintillation counter. The mean value for the two 45-min fluxes was reported as the value for the tissue.

Na-K-ATPase measurements. The effects of varying times and temperature on Na-K-ATPase activity was performed on a commercially available rabbit kidney Na-K-ATPase (Sigma, St. Louis, MO) using the phosphate-determination method of Yoda and Hokin (20). The enzyme was initially dissolved in 10 mM Tris·Cl and 1 mM EDTA, pH 7.4, buffer and diluted 1:10 in 50 mM Tris·Cl, 2.5 mM MgCl₂, and 12.5 mM KCl, pH 7.4. The solution containing enzyme was then heated for a fixed time and at a fixed temperature, and the solution then was allowed to return to 37°C before assessment of enzyme activity. Enzyme activity was assessed by adding 2 mM ATP at 37°C for 1 h, and the reaction was stopped with a solution containing 4% molybdate and 12% perchloric acid. Butyl acetate was added, and the absorbance of the top layer of butyl acetate was measured at 320 nm in a Beckman DU-640 spectrophotometer and compared with that of a blank containing Tris·Cl buffer and ATP, thereby accounting for nonspecific phosphate. The absorbances were converted to phosphate liberated by Na-K-ATPase using a standard curve of KH₂PO₄ concentration vs. absorbance at 320 nm.

Statistics. Statistical significance was determined using Student's t-test for parametric data and the Mann-Whitney test for nonparametric data (injury assessment). All data were reported as the means ± SE. The protocol has been reviewed and approved by the institutional Animal Welfare Committee.
experiments, luminal heat was applied for 5 min at 49°C in an additional four tissues to document the reproducibility of the effects at this modest level of heat exposure (Fig. 2). (In these experiments tissues exposed luminally to 49°C for 5 min were found to have a mean serosal surface tissue temperature of 41°C; this is a reflection of heat transfer from the luminal bath, determined by applying a miniature temperature probe to the serosal surface, n = 4.) In a third set of
experiments, luminal heat up to 70°C was applied for 1 min, and after restoration of 37°C for 5 min, a 1-min period of heat was repeated. This 6-min cycle was repeated six times so that the esophageal epithelium was exposed to heat for 6 min over a 36-min period (Fig. 3). As shown in Figs. 1 and 3, exposure to luminal heat reduces PD, I_{sc}, and R in a temperature-dependent manner, although the temperature thresholds at which reductions occur for R, reflecting epithelial permeability or barrier function, and I_{sc}, reflecting epithelial active ion transport, differ considerably. [PD, being the product of I_{sc} and R (Ohm's law), simply reflects the net change in I_{sc} and R.] Thus it is observed that luminal heat reduced R more readily than I_{sc}. R with a single 5-min exposure rapidly declining below baseline at a temperature of 49°C and I_{sc} remaining uninhibited until the luminal solution temperature reached 60°C (Figs. 1 and 2). (The small, transient, but significant, rise in I_{sc} at 1 min was not reproduced in subsequent experiments and so may in part be artifact). Moreover, once the threshold temperature was reached, luminal heat reduced R rapidly but modestly to its new value and there it remained despite continued exposure to the same level of heat. Additionally, luminal heat-induced reduction in R was a reversible phenomenon, R rising to or toward preheat levels after temperature was restored to 37°C. In contrast, luminal heat, once threshold temperature was reached, inhibited I_{sc} more dramatically and, based on failure of I_{sc} to return toward preheat levels on restoration of 37°C, for the most part irreversibly (Figs. 1–3). Interestingly, tissue morphology was unremarkable at levels in which luminal heat did not inhibit I_{sc}; however, at levels of luminal heat that I_{sc} was inhibited, i.e., ≥60°C whether by single exposure for 5 min or repeated 1-min exposures for 6 min over a 36-min period, blistering of the epithelium was noted (Fig. 4). The plane of cleavage of the blister was localized to the region of the basement membrane, with the basal cells being the roof of the blister and the lamina propria being the floor. Also, in some areas there was evidence of cell edema.

Given the variability in rate and direction of heat transfer and dissipation, the technique of luminal heating left unknown the impact of specific temperatures on the esophageal epithelium. For this reason in some experiments luminal and serosal solutions were simultaneously heated to the same temperature, thereby heat clamping the tissue to a particular level. As shown in Fig. 5, heat clamping tissues over the range of 38–52°C reduced PD, I_{sc}, and R, with I_{sc} being as heat sensitive as R in terms of threshold for effect. Additionally, the impact of heat clamping on I_{sc} was significantly greater than on R at any given temperature, and so much so that, while R was reversibly reduced by 10–15% at 52°C, I_{sc} was irreversibly abolished. Heat clamping to ≥50°C was also associated with epithelial blisters (data not shown).

Because the effect of luminal heat alone or heat clamping the tissue was to inhibit I_{sc} and reduce R, studies were done to clarify the mechanism for the impairment in active transport (I_{sc}) and reduction in barrier function (R). Specifically, because the I_{sc} of esophageal epithelium is predominantly due to active Na transport (15), and enzymes such as Na-K-ATPase are known to be temperature sensitive, we initially sought to directly measure the effect of heat on esopha-
geal Na-K-ATPase activity in homogenized sections of Ussing-chambered heat-exposed epithelium. However, when this proved unreliable technically, we chose instead to use a commercially available rabbit kidney Na-K-ATPase as a model to investigate the effects of heat on Na-K-ATPase activity. As shown in Fig. 6, the enzyme Na-K-ATPase was heat sensitive in a temperature-dependent and time-dependent manner. For example, Na-K-ATPase activity gradually declined as temperature was raised from 37 to 52°C and then declined sharply thereafter until complete inhibition was observed at 60°C. In Fig. 6B, using a temperature of 49°C, it was observed that Na-K-ATPase activity declined from 5 to 30% below baseline over 10 min, whereupon it plateaued and declined no further. Also, to explore the locus for the decline in R on exposure to heat, tissues were heat clamped at 49°C and [14C]mannitol flux measurements were performed and compared with tissues exposed to 37°C. As shown in Table 1, tissues heated to 49°C had a 29 ± 65% decline in R compared with an increase for those at 37°C and more than double the mannitol flux of those at 37°C. Additionally, under these conditions there was no morphological change by light microscopy to the cells of the barrier layer, although there was evidence of subepithelial blistering.

DISCUSSION

Hot beverages are consumed at temperatures as high as 80°C, and, according to (and extrapolated from) the work of De Jong et al. (3), this could expose the esophageal epithelium within the distal esophagus to temperatures as high as 58°C. (De Jong only studied ingestion of hot liquids up to 65°C and found it to elevate distal esophageal temperature as high as 53°C.) In this study, we examined the impact of temperatures covering this range and above on the esophageal epithelium. When the esophageal epithelium was mounted in Ussing chambers and exposed to heat from the luminal side only, mimicking the method of heat delivery on oral ingestion of hot beverages, temperatures of ≥49°C...
reduced PD and R and temperatures of ≥60°C reduced
I_{sc}. Because R is a reflection of permeability and I_{sc} a
reflection of active (ion) transport, these data indicate
that under appropriate circumstances luminal heat can
impair both epithelial barrier and transport functions.
Moreover, once the threshold temperature was reached,
impairment in barrier and transport functions could
occur rapidly and with short exposure times (∼1 min).
(Although esophageal luminal bolus clearance is on the
order 5–10 s per bolus, this time frame may be within
reach given ingestion of repeated boluses of hot liquid
and the absorption and perhaps accumulation of heat
energy transmitted from each bolus to the epithelium).
Interestingly, however, these two functions responded
differently to luminal heat. First, as already noted, they
exhibited different temperature thresholds for impair-
ment by heat, barrier function being the more tempera-
ture sensitive than transport. Second, although having
a lower temperature threshold, barrier function even at
the highest temperatures studied (70°C) stabilized
rapidly and was at worst only moderately impaired by
luminal heat, whereas transport fell more precipitously
once threshold was reached and was abolished at 70°C.
Third, impairment of barrier function by luminal heat
was generally reversible, whereas this was not the case
for the heat-induced inhibition of active transport.

One plausible reason for the differences in response
to luminal heat for barrier and transport functions is

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**Fig. 5.** Effect of both luminal and serosal heat (heat clamping) on PD
(A), I_{sc} (B), and R (C) of rabbit esophageal epithelium in Ussing
chambers. Effects are illustrated for control group of tissues (only
exposed to 37°C) and for those exposed to varying temperatures, and
results are plotted as percent change from baseline value of each
tissue at 37°C. Each temperature plot represents mean value for 3
tissues. Bathing solution and tissue temperatures at various times
are indicated by arrows. After exposure for 5 min at 52°C, bathing
solution temperatures of all tissues were restored to 37°C to monitor
reversibility of heat-induced changes. *P < 0.05 compared with
controls at 37°C. Note as a frame of reference initial PD values were
−11.9 ± 3.6 and −15.0 ± 3 mV, I_{sc} 6 ± 1 and 10 ± 1 µA/cm² and R
1,881 ± 493 and 1,463 ± 294 Ω·cm² for control and heat-stressed
tissues, respectively.

**Fig. 6.** Effect of varying degrees of heat (A) and time of heating (B)
shown as percent change in absorbance compared with baseline
values for activity at 37°C. *P < 0.05 compared with baseline at 37°C;
n = 3.
the location of the cells primarily responsible for these activities. Thus, for example, it would be expected that functions carried out by the cells of the most luminal, i.e., stratum corneum, which come into direct contact with the luminal solution, would be the first to experience the impact of raising luminal temperature. This in fact is the case because barrier function was affected at lower temperatures than transport, and it is the cells of the stratum corneum, through their combination of apical membranes and junctional complex comprised of tight junctions and intercellular glycoconjugates, that are principally involved with barrier function (12). Also, it would be expected that active transport would be less temperature sensitive than barrier function because the cells responsible for this activity are primarily localized within the stratum spinosum and stratum germinativum, two layers physically removed from contact with the heated luminal solution and further protected from luminal heat by heat dissipation-absorption as it traverses the more luminal cells of the stratum corneum (3, 11). Experimental evidence that supports this concept includes both the lower temperature threshold for inhibition of transport with heat clamping and its greater impairment compared with barrier function when tissues were heat clamped, a process that delivered heat to the tissue from the serosal (as well as luminal) side. Under these circumstances, i.e., serosal heating, the transporting cells (and their enzymes, e.g., Na-K-ATPase; see below) in the stratum spinosum and stratum germinativum can come into direct contact with the heated solution or, in the absence of an interposing stratum, have less heat dissipated before contact is made with the cells. Consistent with this interpretation is the observation that barrier function, as reflected in $R_L$, was equally impaired at a given temperature whether heat was delivered only luminally or both luminally and serosally. This suggests that the addition of serosal heat to the tissue had little effect on barrier function because this function is carried out by the more distant cells of the luminal stratum (stratum corneum).

How an increase in tissue temperature produces changes in transport and barrier functions was also investigated. First, because the esophageal epithelium

### Table 1. Effect of heat stress on electrical resistance and transepithelial mannitol flux in Ussing-chambered rabbit esophageal epithelium

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Preheat, $\Omega \cdot \text{cm}^2$</th>
<th>Postheat, $\Omega \cdot \text{cm}^2$</th>
<th>Change from preheat, %</th>
<th>$J_m$, $\mu$mol $\cdot$ h$^{-1} \cdot \text{cm}^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 37°C</td>
<td>5</td>
<td>2,160 ± 138</td>
<td>2,695 ± 146</td>
<td>+25 ± 4</td>
<td>0.0018 ± 0.0006</td>
</tr>
<tr>
<td>Heat stress, 49°C</td>
<td>5</td>
<td>2,161 ± 197</td>
<td>1,529 ± 190*</td>
<td>−29 ± 5*</td>
<td>0.0042 ± 0.0008*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = \text{no. tissues}$. Change from preheat is percent change in resistance from preheat-stress period. Note that in tissues exposed to 37°C, resistance increased, whereas resistance decreased in those under heat stress at 49°C. $J_m$, mucosal-to-serosal mannitol flux. *$P < 0.05$ compared with control.
is primarily a sodium transporting (absorbing) tissue, we investigated the impact of heat on Na-K-ATPase activity (15). When the rabbit kidney Na-K-ATPase was used as a model, it was shown that this enzyme is highly susceptible to inhibition at temperatures used in our experiments. For example, active transport by the cells of the stratum spinosum and stratum germinativum was significantly inhibited with even brief exposure to luminal temperatures of 60°C or to serosal (and luminal) temperatures of 52°C, and at temperatures in this range (50–60°C), it was observed that Na-K-ATPase activity was either markedly inhibited or completely abolished. For this reason, the temperature-induced inhibition in transport in esophageal epithelium was likely a reflection of inhibition of Na-K-ATPase activity and other unmeasured heat-sensitive transport proteins.

Second, because the barrier function of the multilayered esophageal epithelium, as reflected in R, is most readily changed by alterations in its junctional complex (11, 13), we performed transepithelial mannitol fluxes in heat-exposed tissues. As noted in Table 1, the heat-induced reduction in R was accompanied by increases in transepithelial mannitol flux, and under a condition that produced no morphological change (by light microscopy) in the cells of the barrier layer. These results indicate that heat alters esophageal epithelial barrier function by increasing the permeability across the paracellular pathway. Mosely and colleagues (10) also reported similar results with heat stress to cultured monolayers of MDCK cells; that is, heat reversibly increased paracellular permeability without altering tissue transport or viability. Although the mechanism for this occurrence was unknown, among the possibilities considered by them and others were activation of phospholipase C, increased intracellular calcium, altered cytoskeletal elements, altered membrane fluidity, and/or increased production of oxidants (7, 10).

The permeability across the paracellular pathway in rabbit esophageal epithelium is controlled by a series of tight junctions and intercellular glycoconjugates (12), and the present study suggests that the proteins of this barrier are highly sensitive to luminal heat. This sensitivity to luminal heat, however, may be viewed as having both good and bad effects. On the positive side, the ability of luminal heat to increase permeability across the paracellular pathway may serve a protective function, by permitting greater shunting of heat around rather than through the cells of the barrier layers. Alternatively, and on the negative side, our experiments showed that when heat increases the permeability across the paracellular pathway (R reduced), the risk of damage from luminal acidity is also increased. This observation is not surprising because the major path for acid entry into the esophageal epithelium is via this route, and other factors, e.g., hypertonicity and ethanol, that increase paracellular permeability in esophagus similarly increase the damaging effects of luminal acidity (1, 9, 17). That these observations with heat stress and acidity may have clinical relevance is supported by the fact that the risk of esophageal exposure to gastric acid through gastroesophageal reflux is markedly increased under the very same conditions that the esophagus is exposed to luminal heat, i.e., the process of eating and drinking of hot substances (or substances at any temperature).

Finally, luminal heat had little effect on tissue morphology by light microscopy until temperatures reached 60°C. At temperatures of 60°C or higher, subepithelial blisters were noted, as is reported to occur with temperatures above 50°C producing second-degree burns to skin, another stratified squamous epithelium (16). Because the experiments with serosal heat (heat clamping) produced similar blisters at lower temperatures (52°C), blister formation was likely due to a direct effect of heat on the protein fibrils anchoring the epithelium to the underlying lamina propria.

In summary, the present study suggests that human consumption of hot beverages can impair esophageal epithelial structure and function and that the impairment in barrier function increases the risk of damage from contact with refluxed gastric acid. Thus esophageal exposure to high luminal temperature can damage the epithelium both directly and indirectly. These observations in part may help explain the reported association between esophageal disease and human consumption of hot beverages.

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