

HEATING CAUSES A RISE IN THE CONCENTRATION OF INTRACELLULAR SODIUM IN HUMAN CELLS *IN VITRO*

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INTRODUCTION

While the symptoms of heatstroke are well known, the pathophysiological changes resulting in symptoms and death are poorly understood. In particular, those early important subcellular alterations which ultimately lead to impaired whole body homeostasis and organismic heatstroke are virtually unknown.

A rise in intracellular sodium ion concentration ($[Na^+]_i$) was predicted by Hubbard in his "energy depletion mechanism" of heatstroke [1] but such rises have not been clearly shown. In this study, we have found rises in $[Na^+]_i$ at elevated temperatures in isolated human squamous epithelial cells, by measuring changes in the fluorescence of a Na^+ -sensitive fluorophore which had been introduced into those cells.

MATERIALS AND METHODS

Cells: Human cheek squamous epithelial cells were suspended in Hanks Balanced Salt Solution at pH 7.4 (HBSS), incubated for 30 min at 25°C in 5 μ M Sodium Green, and rinsed.

Fluorescence: Ultra-narrow flashes of low energy laser energy (488 nM) were directed through a microscope across the surface of a suitably labeled cell, to scan the cell completely (ACAS 570, Meridian Inst. Co.). Normalized average cell fluorescence was detected with a photomultiplier, stored within an internal computer and rendered into a 2-dimensional pseudo-color image, with the color related to normalized fluorescence intensity. Laser intensity was adjusted so that bleaching of the fluorophore within the cells was less than 2% after 60 scans. It is technically difficult to calibrate absolute fluorescence as a function of sodium concentration inside the cell, and only changes in normalized fluorescence are reported. As a control, the fluorescence of the acid form of Sodium Green was determined in saline at various temperatures.

Cells ($n > 14$) were placed into groups to be heated from 37°C to different final temperatures. They were exposed to a baseline temperature of 37°C for 10 min,

raised to a new temperature, maintained at that new temperature for 20 min, cooled back to 37°C, and maintained at that temperature for 10 min.

RESULTS

No significant changes were seen in cell shape or size upon heating. As a control, addition of Na⁺ to solutions containing the free acid form of Sodium Green, caused fluorescence to rise to a plateau. Unlabeled cells show very low or undetectable levels of fluorescence. However, labeled cells at 37°C, showed a strong fluorescence with a small rise in mean fluorescence over time (drift) but there were no significant differences among groups (Fig. 1). When the temperature was raised there was a transient decrease in fluorescence ($p < 0.05$, Fig. 1, Arrow) followed by a large, gradual rise. At 42 and 43°C, the slope was not constant but gradually increased. When heated cells were cooled to 37°C, the fluorescence did not fall, but at temperatures $\geq 43^\circ\text{C}$, continued to rise at high rates.

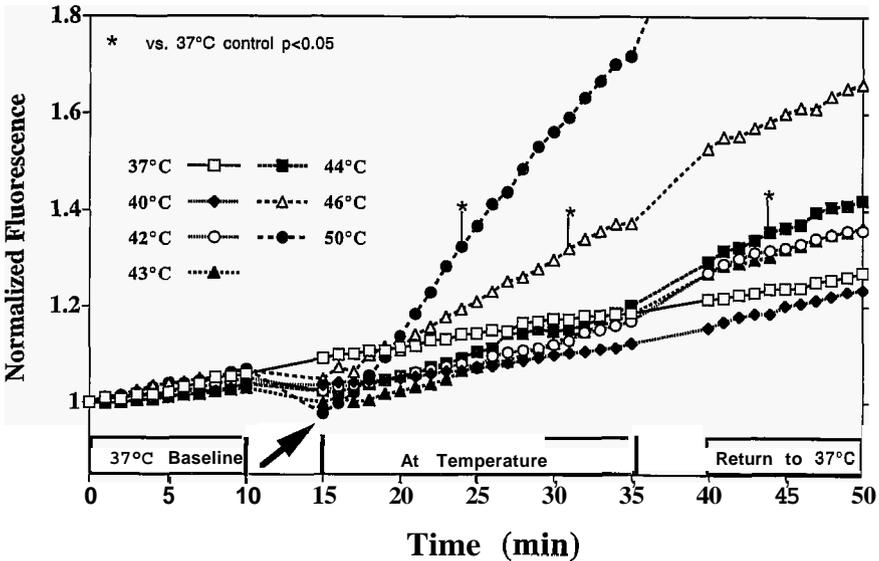


Figure 1. Fluorescence of human squamous epithelial cells *in vitro* labeled with a sodium-sensitive dye (Sodium Green), heated in one step to the indicated temperatures. When the cells were heated, first they showed a transient fall in fluorescence (Arrow) followed by a rise. Upon cooling to 37°C, the fluorescence did not return to baseline but increased still further.

CONCLUSIONS

In previous studies on heatstroke in animals and humans, little or no change was noted in plasma Na^+ concentrations, suggesting that no significant change occurred in $[\text{Na}^+]_i$ [2]. However, in attempting to show small intracellular changes against a background of high extracellular $[\text{Na}^+]_o$, those studies utilized less-sensitive analytical techniques and often used indirect methods [3]. The ACAS system employed here expresses Na^+ concentrations through changes in relative fluorescence within individual cells, one cell at a time, against a very low background, and is more sensitive to local changes in Na^+ .

Heating. $[\text{Na}^+]_i$ depends upon a steady-state relationship between the rates of Na^+ influx and efflux. Hyperthermia increases the rate of Na^+ influx.

At 37°C and under normal ionic concentrations, Na^+ efflux is due almost exclusively to the Na^+/K^+ -ATPase pump. Its pumping rate increases with both a rise in temperature and a rise in $[\text{Na}^+]_i$. Therefore, moderate rises in temperature, cause Na^+ both to enter cells more rapidly and be pumped out more rapidly. If the Na^+ efflux is greater than its influx then $[\text{Na}^+]_i$ falls. This can account for the initial drop in $[\text{Na}^+]_i$ upon heating (Fig. 1, Arrow). The eventual elevated slope of fluorescence, however, suggests that Na^+ influx rapidly becomes greater than its efflux. Above 44°C , the upwardly-curving slope of fluorescence suggests that the Na^+/K^+ -ATPase pump is gradually being thermally inactivated.

Cooling. Previous studies by other groups have shown some activity of the Na^+/K^+ -ATPase pump at temperatures as high as 45°C . If this so, then upon cooling back to 37°C from 44°C , there should be little change in Na^+ influx from its baseline value. At higher temperatures, however, and if only the Na^+/K^+ -ATPase pump had been irreversibly denatured by heat, then upon cooling back to 37°C , the rate of rise of $[\text{Na}^+]_i$ should be much higher than its 37°C value because the Na^+ pump is no longer functioning. Such an effect is seen in Figure 1 at 50°C .

Small rises in $[\text{Na}^+]_i$ can lead to large rises in Ca^{++} influx via the $\text{Na}^+/\text{Ca}^{++}$ exchanger because the Ca^{++} driving force depends upon the third power of $[\text{Na}^+]_i$ [4]. Therefore, heating can be expected to eventually activate Ca^{++} -dependent pathways, possibly leading to the production of immune modulators [5,6].

Energy-Depletion Model. In this model the acidosis of hyperthermia activates the Na^+/H^+ exchanger, and, if the temperature is near the maximum rate of the Na^+/K^+ ATPase pump, then $[\text{Na}^+]_i$ would rise, as seen here.

If the rise in $[Na^+]_i$ described here extends to nerve cells, then according to the Nernst equation, the magnitude of action potentials would be reduced. This, in turn, could affect physiological mechanisms, psychological function and physical coordination, and therefore may be a risk factor as well as be a component of the pathophysiology of heatstroke. High fever would be expected to show similar sequelae.

In summary, heating caused a rise in $[Na^+]_i$ in human squamous epithelial cells which persisted in time, and was not rapidly reversed with cooling.

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