INTERRACELLULAR pH IN GASTRIC AND RECTAL TISSUE POST CARDIAC ARREST

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1. INTRODUCTION

Hypoperfusion to the gut during cardiac arrest is an important clinical problem. The inability to control pH during metabolic stress, e.g. ischemia, leads to the disruption or halting of processes vital to balancing cellular metabolism. Alterations in cellular pH have been linked to changes in intramucosal permeability, which may result in the leakage of inflammatory mediators or bacteria, or both, into the systemic circulation and contribute to the development of organ failure, shock, or death.

A strong relationship exists between energy metabolism and cellular acid-base balance. Acidosis during ischemia is likely due to glycolytic accumulation of CO₂, lactic acid, and H⁺. Tissue P CO₂ is a recognized clinical marker of perfusion failure, resulting from a variety of conditions (hemorrhagic shock, sepsis, trauma) that arise when tissue O₂ requirements can no longer be met and anaerobic metabolism is initiated. The link between gastric mucosal hypercarbia and intracellular pH (pHi) has not been established. The relationship between gut tissue P CO₂ and pHi is clinically important since cellular/tissue pH monitoring technologies are not available at the bedside.

To our knowledge, gut pHi under hypoxic conditions has not previously been reported. This study was undertaken, then, to: 1) define baseline pHi of stomach and rectum, two clinically useful monitoring sites; 2) evaluate changes in pHi at these sites during ischemia; 3) identify differences in pHi in the gut layers; 4) compare pHi to mucosal P CO₂; and finally 5) estimate the buffering of pHi in the stomach and rectum and compare these values with arterial bicarbonate (HCO₃⁻).

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2. METHODS

2.1 Surgical Preparation

The surgical protocols for anesthesia, cannulation of vessels, and insertion of P$_{CO2}$ electrodes in rats has been previously described. While the abdominal cavity was open for instrumentation of the stomach, a small catheter (IP catheter) was inserted into the peritoneal cavity and the cavity was closed with sutures.

2.2 Experimental Protocol

Post-instrumentation, rats were injected via the IP catheter with 3 mL of 2% Neutral Red Dye (Sigma Chemical). The rat was repositioned several times during the first 5 minutes post-IP infusion to distribute dye to the gut tissue. All animals were stabilized for 30 minutes in order for mean arterial pressure to return to baseline. Arrest was induced by an intra-atrial injection of norcuron (.1-.2 mg/kg) followed by a potassium chloride bolus (0.5M/L; 0.12 mL/100 gm of body weight). Control animals remained under anesthesia while the stomach and rectum were harvested. Tissues were immersed in liquid nitrogen, and stored at -80°C. The rats were then euthanized by cutting the abdominal aorta. Organs from rats in the arrest group were similarly harvested and frozen.

2.3 pH$i$ Analysis

The pH$i$ is a measure of the acid-base status of cells within a specified area. The pH$i$ was determined by a reflectance histophotometric imaging technique using the absorption dye neutral red. Tissue was mounted on a cryotome chuck and maintained at -25°C. An unstained tissue blank was mounted next to each tissue block prior to photographing the sample monochromatically at 550 nm and 450 nm. The pH$i$ was calculated as: pH$i$ = -1.3(OD 550/OD 450) +10.5. Transmittance was determined by dividing pixel values from the 550 and 450 images by the 100% transmittance level obtained from the blank. Using Image Pro Plus (4.0 Media Cybernetics) images were processed by first converting them to floating point images, determining a ratio, and comparing values to a calibration curve. Grayscale tissue images were used to identify the area of interest (AOI) and these AOIs were identified within the pixel images. Mean AOI pH$i$ values were determined after eliminating values outside of the defined dye range, 6-8 units. Each tissue was sectioned 3-4 times and a separate determination was made of the pH$i$ for the whole tissue (all layers within the area) and for layers (mucosal, submucosal, muscularis mucosa).

2.4 Data Analysis

Statistical analysis was performed using JMP IN computer software (Version 4, SAS Institute). For each rat 3 to 4 slices of the same tissue were analyzed. The average pH$i$ (mean ± SEM) for whole tissue and for layers, was determined for each slice by taking the average of pixel values within the AOI that fell within the pH range of 6-8 units. The AOI was drawn to include the largest possible area of each tissue sample. Because the same spot on each tissue was not sampled (e.g., the fundus of the stomach was not sampled consistently), tissue and group were treated as random effects in the model, thereby increasing the number of observations included in the analysis (whole tissue, n=99; layers, n=283). To evaluate changes in the pH$i$ by site (rectal vs. gastric) and group, ANOVA was used. To compare pH$i$ by layer over the experimental protocol a