Regional Changes in Intracellular pH Determined by Neutral Red Histophotometry and High Energy Metabolites During Cardiac Arrest and Following Resuscitation in the Rat

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Intracellular pH was determined by neutral red color histophotometry in cerebral tissue from rats subjected to 10 minutes of cardiac arrest and from rats that had recovered for 1 and 6 hours following 8-10 minutes of total cerebral ischemia (TIA). Tissue concentrations of ATP, lactate and glucose were measured corresponding to the pH determinations. As expected, tissue ATP was depleted while tissue lactate was markedly elevated after 10 minutes of ischemia without reflow in the cerebral cortex, striatum and hippocampus. However, both metabolites were near control following 1 and 6 hours of recovery in all three regions. Tissue glucose was not significantly different from control following 1 and 6 hours of reperfusion. During ischemia, the intracellular pH dropped to 6.5-6.7 in all three regions (p<0.05). But, since the initial pH of the hippocampus was 7.79 while that of the cerebral cortex and striatum was approximately 7.02, the net drop in pH of the hippocampus was greater than in the other two regions. Following 1 hour of reperfusion, a trend towards tissue alkalosis was observed in the cerebral cortex and striatum.

KEY WORDS: ischemia; lactate; intracellular pH; tissue acidosis.

INTRODUCTION

During cardiac arrest, tissue lactate concentrations rise as a consequence of anaerobic glycolysis. The enzymatic reduction of pyruvate to lactate results in the concomitant, stoichiometric generation of protons, leading to a decrease in tissue pH. The degree of tissue

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acidosis during ischemia may limit the recovery of ischemic tissue following resuscitation (Siesjö, 1988). In animal studies, increased glucose load before ischemia resulted in decreased return of cellular energy metabolites (Welsh et al., 1980; Rehncrona et al., 1981), increased histological damage (Kalimo et al., 1981), decreased functional recovery (Pulsinelli et al., 1982; Siemkowicz, 1981), increased brain edema and seizure activity (Warner et al., 1987), greater perfusion deficits (Ginsberg et al., 1980) and greater impairment of glucose utilization (Ginsberg et al., 1980) in the recovery phase following cerebral ischemia. This increased damage was attributed to more severe tissue acidosis as a consequence of increased substrate for anaerobic glycolysis in hyperglycemic animals.

To begin to better understand the mechanism which links lactate production to ischemic tissue damage, an estimate of intracellular pH from tissue with known lactate concentration will be necessary. In this study, pH_i was estimated during and following recovery from cardiac arrest induced total cerebral ischemia (TCI) using the pH sensitive vital dye, neutral red. The color of neutral red changes in response to subtle changes in pH. This color shift can be measured and used to estimate intracellular pH (LaManna and McCracken, 1984). In addition, tissue ATP, lactate and glucose concentration were measured in these same locations.

**MATERIALS AND METHODS**

Neutral red (purified, >90% dye content) was purchased from Sigma. Enzymes for the metabolite assays were purchased from Sigma or Boehringer-Mannheim.

**Animal Preparation**

Male Wistar rats (300-350 g) were purchased through our animal facilities and housed on a 12 hour light-dark diurnal cycle. The rats had free access to food and water except for 12-24 hours prior to the experiment when food was withheld to provide a moderate, stable, plasma glucose concentration. All procedures were reviewed by our animal care and use committee and were approved by the committee before implementation.

The rats were anesthetized with methohexital sodium (Brevital, 60 mg/kg, i.p.). The right atrium and ventral tail artery were cannulated and the animals were allowed to recover from anesthesia for at least 3 hours in a plastic restraint. Total cerebral ischemia was induced using a cardiac arrest model (Blomqvist and Wielock, 1985) as modified by Crumrine and LaManna (1991). Briefly, the arterial catheter was connected to a pressure transducer and the systemic arterial blood pressure was monitored on a chart recorder (Gould Inc., Cleveland, Ohio). Cardiac arrest was induced by the rapid injection of d-tubocurare followed immediately by 0.5 M ice cold KCl (0.15 ml/100 g). Non-resuscitated animals were frozen in situ after 10 minutes. Reperfused animals were removed from the plastic restraint after 1 minute and put in supine position. They were orotracheally intubated and returned to a prone position. The tracheal tube was attached to a small animal respirator (Harvard Apparatus, South Natick, MA). The ventilator was activated after 7 minutes of ischemia.