Hyperglycemia Alters Expression of Cerebral Metabolic Genes after Cardiac Arrest

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Background: Survivors of cardiac arrest often experience neurologic deficits. To date, treatment options are limited. Associated hyperglycemia is believed to further worsen the neurologic outcome. The aim with this study was to characterize expression pathways induced by hyperglycemia in conjunction with global brain ischemia. *Methods:* Pigs were randomized to high or normal glucose levels, as regulated by glucose and insulin infusions with target levels of 8.5-10 mM and 4-5.5 mM, respectively. The animals were subjected to 5-minute cardiac arrest followed by 8 minutes of cardiopulmonary resuscitation and direct-current shock to restore spontaneous circulation. Global expression profiling of the cortex using microarrays was performed in both groups. *Results:* A total of 102 genes differed in expression at $P < .001$ between the hyperglycemic and the normoglycemic pigs. Several of the most strongly differentially regulated genes were involved in transport and metabolism of glucose. Functional clustering using bioinformatics tools revealed enrichment of multiple biological processes, including membrane processes, ion transport, and glycoproteins. *Conclusions:* Hyperglycemia during cardiac arrest leads to differential early gene expression compared with normoglycemia. The functional relevance of these expressional changes cannot be deduced from the current study; however, the identified candidates have been linked to neuroprotective mechanisms and constitute interesting targets for further studies.

Key Words: Cerebral—ischemia-reperfusion—gene expression—glucose—hyperglycemia—microarray—pigs.

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Introduction

Cerebral ischemia after cardiac arrest (CA) is common and life-threatening, and those who survive often experience significant cerebral dysfunction. The treatment options are limited and aim at optimizing cerebral perfusion and oxygenation complemented with induced therapeutic hypothermia.[1,2] Concomitant hyperglycemia is frequently perceived after CA[3] and worsens neurologic outcome.[4,5] Glycemic control is therefore an important clinical goal[6] among other efforts to attenuate the neurologic damage after CA.

The development of neuronal injury following global ischemia is likely exacerbated by the reperfusion that follows once blood flow is restored to the ischemic area.[7] The absence of cerebral blood flow initiates a cascade of molecular events such as anaerobic glycolysis, lactate acidosis, free radical production, altered cell signaling, inflammatory processes, and activation of gene expression.[8] Paradoxically, when replenishment of oxygen occurs, the cerebral tissue damage can be augmented by means of increased production of reactive oxygen and nitrogen species, that is, oxidative stress.[9] Concomitant acute hyperglycemia further aggravates the ischemia-reperfusion injury in synergy with mediators of oxidative stress[10]; however, the causative upstream molecular mechanisms are unknown.

In a previous report from our laboratory, a subtle but significant increased level of S100β was identified in hyperglycemic pigs following 5 minutes of CA.[11] The aim of the current study was to improve the understanding of the pathogenesis behind how concomitant hyperglycemia can augment the injury after cerebral ischemia. The results could generate hypothesis for the development of new therapeutic options to prevent or minimize secondary neuronal damage after cerebral ischemia. To that end we performed a global transcriptome analysis of brains from hyperglycemic and normoglycemic pigs after CA.

Methods

Animals and Surgery

The experimental procedure was approved by the Uppsala Ethical Committee for Animal Research (C19/8) and has been described.[11] In brief, 12 triple-breed male piglets, obtained from a single provider (10-12 weeks old, 22-29 kg), were randomized to high or normal glucose levels, as regulated by glucose and insulin infusions with target levels of 8.5-10 mM (hyperglycemic group [HG]) and 4-5.5 mM (normoglycemic group [NG]). There was no weight difference between the groups. The animals were acquired from a local farmer and transported individually to the operation facility, 1 animal per day.

Anesthesia was induced and maintained as described.[11] An intramuscular injection of tiletamine-zolazepam (Zoletil, Reading Laboratories, Carros, France, 6 mg/kg), xylazine (Rompun, Reading Laboratories, 2.2 mg/kg), and atropine (.04 mg/kg) was given, after which vein catheters were placed in the ears for intravenous anesthesia and fluid administration. Airway access was through a tracheotomy after 20 mg of morphine, and the animals were mechanically ventilated (Servo I, Macquet, Solna, Sweden) with a respiratory frequency of 25 per minute using volume-controlled mode with a FiO2 of .30, I-to-E ratio of 1:2. Tidal volumes were adjusted to maintain normocapnia of 5.0-5.5 kPa. An infusion of pentobarbital (Pentothal, 8 mg/kg/h), morphine (.5 mg/kg/h), and pancuronium bromide (Pavulon, Reading Laboratories, .25 mg/kg/h) was used to maintain anesthesia. Volume loss through ventilation and diuresis was compensated for by Ringer's acetate (30 mL/kg) during the first hour; thereafter, bolus doses of 100 mL were given on demand to treat hypovolemia (mean arterial pressure < 70 mm Hg) along with tachycardia and increased respiratory variations in blood pressure.

Under anesthesia, CA was induced by applying alternating current, resulting in ventricular fibrillation allowed to continue for 5 minutes followed by 8 minutes of cardiopulmonary resuscitation, then direct-current defibrillation was applied to restore spontaneous circulation.

Hemodynamic parameters were recorded. Blood was sampled for glucose and protein S100β analyses. These results have been presented.[11]

After 180 minutes of observation period, the piglets were euthanized under deep anesthesia and the brain was removed and stored in –70°C until usage.

RNA Preparation

Isolation of total RNA from 12 pigs (HG = 6 and NG = 6) was performed by quickly dissecting approximately 100 mg of the frontoparietal cortex, and instantly putting the brain tissue in homogenizing buffer containing β-mercaptoethanol according to manufacturer's protocol (Qiagen Inc., Valencia, CA). The tissue was then immediately homogenized using a Polytron homogenizer and total RNA isolated by RNeasy Mini kit (Qiagen) with absorbance determined at 260 and 280 nm.

Microarray Expression Analysis

RNA quality was evaluated using the Agilent 2100 Bioanalyzer system (Agilent Technologies Inc, Palo Alto, CA). A total of 250 ng of total RNA from each sample was used to generate amplified and biotinylated sense-strand cDNA from the entire expressed genome according to the GeneChip WT PLUS Reagent Kit User Manual (P/N 703174 Rev. 1, Affymetrix Inc., Santa Clara, CA). GeneChip ST Arrays (GeneChip Porcine Gene 1.0 ST Array) were hybridized for 16 hours in a 45°C incubator, rotated at 60 rpm. According to the GeneChip Expression Wash, Stain and Scan Manual (P/N 702731 Rev. 3, Affymetrix Inc., Santa Clara, CA), the arrays were then washed and stained using the Fluidics Station 450 and finally scanned using the GeneChip Scanner 3000 7G.