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BRAIN INJURY FOLLOWING REPETITIVE APNEA IN NEWBORN PIGLETS

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1. INTRODUCTION

In spite of the vast literature on the pathophysiology of hypoxia, the mechanism(s) by which repetitive apnea affects the metabolic pathways that sustain the normal processes of cell survival and cell death in the brain. More importantly, there is a need to elucidate the identity of the parameters that, alone or in combination, determine the pathogenic outcome of the apneic insults. Our early studies show that, in newborn piglets, repetitive apnea causes a progressive decrease in cortical oxygenation[1]. In the present studies, the changes in brain oxygenation were correlated with changes in extracellular dopamine, hydroxyl radicals and with neuronal injury. The studies were done on striatum, "selectively vulnerable" dopaminergic region of the brain, which is shown to be uniquely susceptible to recurrent hypoxic events.

Dopamine is widely believed to play a critical role in the physiopathology of brain function. Dopaminergic system of the striatum of a newborn piglet's brain has been shown to be very sensitive to the local oxygen pressure[2-5]. It has been suggested that release of dopamine during ischemic/hypoxic conditions may play a major role in mediating neuronal damage, particularly in the striatum. Massive increase in extracellular dopamine in the striatum has been reported to occur in different models of cerebral ischemia/hypoxia. There is evidence that this increase in extracellular dopamine may promote or accentuate brain damage during reoxygenation by increase of level of free radicals. Both spin trapping[6,7] and direct[8] electron paramagnetic resonance measurements have indicated that reactive oxygen metabolites are formed in the first minutes of reperfusion after cerebral ischemia. Free radicals are probably the major cause of endothelial damage and brain edema after asphyxia. The newborn infant, particularly the preterm infant, is thought to be prone to tissue damage from oxidative stress because of reduced total antioxidant capacity (see rev.[9]). The brain is very susceptible to damage from free radicals because of its high concentration of polyunsaturated fatty acids (see revs. [10-12]), low activity of catalase, superoxide

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dismutases and glutathione peroxidase, high activity of xanthine oxidase[10, 13], high levels of iron[14] and oxidizable catecholamines[10], and low content of transferrin[15] etc.

In the present studies the level of ortho-tyrosine in striatum was used as a measure of *in vivo* hydroxyl radical production. Fluoro-Jade (FJ) was use to determine neuronal injury following repetitive apnea. FJ sensitively and reliably stains the cell bodies, dendrites, axons and axon terminals of degenerating neurons but does not stain healthy neurons, myelin or vascular elements of neuropil[16]. FJ was shown to mark apoptotic cells in mice[17] and neurons injured by methamphetamine[18], excitotoxic (kainic acid) and neurotoxic (trimethyltin) compounds[19].

2. METHODS

2.1. Animal Model

Newborn piglets, age 3-5 days, were used for all studies. Anesthesia was induced with halothane (Halocarbon Laboratories, Augusta, SC; 4% mixed with 96% oxygen), and 1.5% lidocaine-HCl (Abbott Laboratories, North Chicago, IL) was used as a local anesthetic. Halothane was withdrawn entirely after the tracheotomy, pancuronium was used to induce respiratory paralysis (Gensia Pharmaceuticals, Irvine, CA; 1.5 mg/kg). Fentanyl-citrate (Elkins-Linn, Inc., Cherry Hill, NJ) was intravenously injected at 30 µg/kg, and the animals were mechanically ventilated with a mixture of oxygen and 0.5% isoflurane (Baxter Healthcare Corp., Dearfield, IL). The femoral artery and femoral vein were then cannulated and the piglet was maintained on a D_5LR infusion with 10 mcg/kg/hr of Fentanyl-citrate throughout the experiments. The head was placed in a Kopf stereotaxic holder and an incision was made along the circumference of the scalp. The scalp was removed to expose the skull and a hole approximately 5 mm in diameter was made in the skull over one parietal hemisphere for measuring the oxygen pressure. In all experiments, the blood pressure, body temperature and respiratory rate were monitored. The blood pH, $PaCO_2$ and PaO_2 were measured using a Chiron/Diagnostics Rapidlab 800 blood gas machine.

2.2 Repetitive Apnea Model

Each animal underwent 10 episodes of apnea and recovery. Apnea was initiated by disconnecting the animal from the ventilator and completed by reconnecting it to the ventilator. The apneic episodes were terminated 30 sec after the heart rate reached the bradycardic threshold of 60 beats per minute. This simulates the delayed response time to resuscitation. The normal heart rate range for a piglet is between 140-190, similar to that of human infants. The piglets had a recovery period of 10 minutes on mechanical ventilation between periods of apnea.

2.3 Measurement of Extracellular Dopamine

The extracellular level of dopamine in striatum was measured as described in earlier publications[2,3,5].. The microdialysis samples were collected at the end of every apneic episode. Identification and quantitation of dopamine was by comparison with chromatograms of standard solutions. The values for the level of dopamine in the dialysate are presented after correction for relative recovery by the microdialysis probe.