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Acetylcholine esterase activity and behavioral response in hypoxia induced neonatal rats: Effect of glucose, oxygen and epinephrine supplementation

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ABSTRACT

Brain damage due to an episode of hypoxia remains a major problem in infants causing deficit in motor and sensory function. Hypoxia leads to neuronal functional failure, cerebral palsy and neuro-developmental delay with characteristic biochemical and molecular alterations resulting in permanent or transitory neurological sequelae or even death. During neonatal hypoxia, traditional resuscitation practices include the routine administration of 100% oxygen, epinephrine and glucose. In the present study, we assessed the changes in the cholinergic system by measuring the acetylcholinesterase (AChE) activity and the behavioral responses shown by hypoxia induced neonatal rats and hypoxic rats supplemented with glucose, oxygen and epinephrine using elevated plus-maze and open-field test. The acetylcholine esterase enzyme activity showed a significant decrease in cerebral cortex, whereas it increased significantly in the muscle of experimental rats when compared to control. Hypoxic rats supplemented with glucose, glucose and oxygen showed a reversal to the control status. Behavioral studies were carried out in experimental rats with elevated plus-maze test and open-field test. Hypolocomotion and anxiogenic behavioral responses were observed in all experimental rats when compared to control, hypoxic rats supplemented with glucose, glucose and oxygen. Thus, our results suggest that brain damage due to hypoxia, oxygen and epinephrine supplementation in the neonatal rats cause acetylcholine-neuromuscular-defect leading to hypolocomotion and anxiogenic behavioral response. Glucose and glucose with oxygen supplementation to hypoxic neonates protect the brain damage for a better functional status in the later life.

1. Introduction

Hypoxia is one of the most common reasons for neonatal morbidity and mortality, causing reduced oxygen supply to the vital organs (Low et al., 1993) and injury to the developing brain (Deliv-oria-Papadopoulos & Mishra, 2000; Li & Jackson, 2002; Rodrigo, Fernandez, Serrano, Peinado, & Martinez, 2005; Xu, Chi, & Row, 2004). The response of central nervous system to oxygen deprivation/hypoxia are vital in revealing mechanisms that participate in coordinated behavior of respiratory and vasomotor activities (Acker & Acker, 2004; Solomon, 2000). Disruption of the cholinergic innervations during postnatal development results in delayed cortical neuronal development and permanent changes in cortical cytoarchitecture and cognitive function (Angela & Russell, 2004).

Approaches to prevent/treat cerebral hypoxic damage in neonates (Tuor, Del Bigio, & Chumas, 1996) are important for neonatal intensive care unit. During neonatal hypoxia, traditional resuscitation practices include the routine administration of 100% oxygen, epinephrine and glucose (Berg et al., 1996; Biarent et al., 2005; Brambrink, Ichord, Martin, Koehler, & Traystman, 1999; Burchfield, Preziosi, Lucas, & Fan, 1993). But there has always been considerable concern about the potential adverse effects of using 100% oxygen and epinephrine during resuscitation of infants at birth. It was reported that hyperoxia triggers diffuse apoptosis in the immature rodent brain peaking at 3–7 postnatal days, a particularly vulnerable period corresponding to the brain growth spurt of rodents (Sola, Rogido, & Deulofeut, 2007). In our previous study, we reported glutamate mediated neurotoxicity in hypoxic neonates supplemented with 100% oxygen and epinephrine, where as glucose administration protected the neurons from glutamate neurotoxicity (Paulose, Finla, Reas Khan, & Amee, 2007).

Cholinergic systems in the CNS play an important role in the learning and memory (Lena & Changeux, 1998; Levey, Edmonds, Koliatsos, Wiley, & Heilman, 1995). Acetylcholine (Ach) is required for cholinergic neurotransmission in the central and peripheral nervous systems (Goodman & Soliman, 1991). AChE activity has been used as a marker for cholinergic activity. AChE plays a very important role in the ACh-cycle, including the release of ACh (Kouninio-tou-Krontiri & Tsakiris, 1989). The duration of action of ACh at the synaptic clefts is critically dependent on AChE activity (Cooper,

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Bloom, & Roth, 2003). Acetylcholine plays an integral role in normal muscle functions, motor activity, attention, fear, anxiety and learning (Ferry, Roozendaal, & McGaugh, 1999; Fujimaki, Morinobu, & Duman, 2000). Memory process is controlled through cholinergic interactions with their neurotransmitters (Ohno, Kobayashi, Kishi, & Watanabe, 1997). Comparison of the effects of frontal cortex lesions between rat, monkey and human has shown that the remarkably similarity in their behavior (Kolb, 1984). Hypoxic injury is associated with enhanced release of glutamate (Abramov, Scorziello, & Duchen, 2007). This insult sets into motion complex cellular physiological pathways that result in deficits in several neurotransmitter systems, including cholinergic transmission (Chleide & Ishikawa, 1990; Gibson & Duffy, 1981). Hence, studies related to alterations in AChE in hypoxic rodent neonates will be helpful to evaluate their influence on the possible cognitive and anxiogenic behaviour occurring during later stages of life.

Elevated plus-maze test has been widely used to test anxiogenic or anxiolytic properties, memory impairment and general motor activities of experimental animals. The anxiogenic-like activities of experimental rats were manifested in the elevated plus-maze test by following parameters: (a) lower percentage of time spent by the rats in the open arms, (b) lower percentage of entries made into the open arm, (c) risk assessments were done by stretched posture attempt and direct exploration attempt, (d) displacement activities were measured by head dipping and grooming attempts (Shah & Treit, 2004). The locomotory and exploratory activity of experimental animals were measured by crossing attempts, walking time, episodes of rearing, head sniffing and washing attempts made in response to open-field test (Halina & Roza, 2006).

The present study aims at identifying the link between hypoxic stress, AChE activity and behavioral responses of hypoxic neonatal rats and those supplemented with glucose, oxygen and epinephrine. AChE activity was measured in the cerebral cortex and muscle of experimental rat and the behavioral responses of these experimental rats were studied using elevated plus-maze and open-field test. The biochemical alterations in AChE activity restraints the behavioral changes observed in the experimental animals. The altered ACh release during hypoxic condition and on supplementation with 100% oxygen and epinephrine causes defective locomotory and anxiogenic behavioral response during later life. Our experiments were carried out to understand the effective sequence of administration of oxygen, epinephrine and glucose to be followed for improved resuscitation in neonatal care.

2. Materials and methods

All biochemicals used in the present study were of analytical grade and purchased locally.

2.1. Induction of hypoxia in neonatal rats

Wistar neonatal rats of 4 days old weighing 6.00–7.50 g were used for the experiments. Induction of hypoxia and supplementation of glucose, oxygen and epinephrine were done according to the procedure of Paulose et al. (2007). Experimental animals were grouped as follows: (i) control neonatal rats were given atmospheric air (20.9% oxygen) for 30 min (C); (ii) hypoxia was induced by placing the neonatal rats in a hypoxic chamber provided with 2.6% oxygen for 30 min (Hx); (iii) hypoxic neonatal rats were injected 10% dextrose (500 mg/kg body wt) intra-peritoneally (ip) immediately after induction of hypoxia (Hx + G); (iv) hypoxic neonatal rats were supplied with 100% oxygen for 30 min immediately after induction of hypoxia (Hx + O); (v) hypoxic neonatal rats were injected 10% dextrose (500 mg/kg body wt) ip immediately after induction of hypoxia and then treated with 100% oxygen for 30 min (Hx + G + O); (vi) hypoxic neonatal rats were injected epinephrine (0.1 μg/kg body wt) ip immediately after induction of hypoxia and then treated with 100% oxygen for 30 min (Hx + E + O); (vii) hypoxic neonatal rats, 10% dextrose (500 mg/kg body wt) and epinephrine (0.1 μg/kg body wt) were injected ip immediately after induction of hypoxia and then treated with 100% oxygen for 30 min (Hx + G + E + O). All experiments were carried out at room temperature. All groups of neonatal rats were maintained under optimal conditions, 12 h light and 12 h dark for one week. Rats were weighed and sacrificed by decapitation. The cerebral cortex was dissected out quickly over ice according to the procedure of Glowinski and Iversen (1966). The thigh muscles were dissected and the tissues were stored at −70 °C for all experiments. All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines and care was taken to minimize the suffering of the experimental rats.

The role of acetylcholine was measured in the cerebral cortex and muscle of experimental rats one week after the induction of hypoxia and supplementation of glucose, oxygen and epinephrine. For behavioral study, the experimental rats were retained for next 21 days (1 month old rats) for elevated plus-maze test and open-field test.

2.2. Acetylcholine esterase activity

Acetylcholine esterase activity in cerebral cortex and muscle were done using the spectrophotometric method of Ellman, Courteney, Andres, and Featherstone (1961). Homogenate (10%) of cerebral cortex and muscle were prepared in 30 mM sodium phosphate buffer, pH 8.0, containing 1% Triton X 100 to release the membrane bound enzyme and it was centrifuged at 12,500g for 30 min at 4 °C. Acetylthiocholine iodide of different concentrations, 0.25–0.5 mM for cerebral cortex and 0.1–0.8 mM for muscle were used as substrate. The mercaptan formed as a result of the hydrolysis of the ester reacts with an oxidizing agent 5,5′-dithiobis (2-nitrobenzoate) read at 412 nm in Shimadzu UV1700 spectrophotometer.

Protein was measured by the method of Lowry, Rosenbrough, Farr, and Randall (1951) using bovine serum albumin as standard.

2.3. Behavioral study

2.3.1. Elevated plus-maze test

The elevated plus-maze is a widely used animal model of anxiety that is based on two conflicting tendencies; the rodent's drive to explore a novel environment and its aversion to heights and open spaces. The elevated plus-maze (constructed of gray colored wooden planks) consisted of four arms arranged in the shape of a cross. Two arms had side walls and an end wall ("closed arms")—the two other arms had no walls ("open arms"). The open arms were surrounded by small ledges to prevent the animal falling from the maze. The maze was fastened to a light-weight support frame. Thus "anxious" animals spent most of the time in the closed arms while less anxious animals explored open areas longer.

2.3.1.1. Procedure. Animals were placed individually into the center of elevated plus-maze consisting of two open arms (38L × 5W cm) and two closed arms (38L × 5W × 15 Hcm), with a central intersection (5 cm × 5 cm) elevated 50 cm above the floor. Behavior was tested in a dimly lit room with a 40 W bulb hung 60 cm above the central part of the maze. The investigator sitting approximately 2 m apart from the apparatus observed and detected the movements of the rats for a total of 5 min. The experimental procedure was similar to that described by Pellow, Chopin, Files, and Briley (1985). During the 5 min test period, the following parameters were measured to analyze the behavioral changes of the experimental rats using elevated plus-maze: open arm entry,