Introduction

Hypoxia is one of the major causes of damage to the foetal and neonatal brain. Newborn babies are frequently exposed to hypoxia and ischemia during the perinatal period as a result of stroke or problems with delivery or respiratory management after delivery (William et al., 2005). Although systemic and cerebrovascular physiologic factors play an important role in the initial phases of hypoxic-ischemic injuries, the intrinsic vulnerability of specific cell types and systems in the developing brain is more important in determining the effect of damage and functional disability. In mild cases, hypoxia causes inattentiveness, poor judgment and motor in-coordination. If the lack of oxygen to the brain is limited to a very brief period of time, damage with varying levels reverse to function, depending on the extent of injury. Cerebral hypoxia refers to a condition in which there is decrease in oxygen supply to the brain in spite of adequate blood flow. Drowning, strangling, choking, suffocation, cardiac arrest, head trauma, carbon monoxide poisoning and complications of general anesthesia create conditions that lead to cerebral hypoxia. Hypoxia affects the central nervous system (CNS) both functionally and morphologically (Flynn et al., 1977; Nelson & Lynch, 2004).

Foetuses, that experience injuries in the womb, premature births and birth complications, live rest of their lives in fear of growth and development (Mark, 1993). The acute interruption or reduction of cerebral blood flow, induced by several factors and clinical pathologies, reduces available oxygen to the nervous system. As the placenta stops growing during the final months of pregnancy, it becomes tough and fibrous, causing blood vessels degeneration, making the foetus more susceptible to hypoxia (Heinz, 1970, Hein & Kobilka 1995). Furthermore, the weight of the foetus
pressing down into the pelvis can compress blood vessels supplying the placenta, producing additional placental failure (Briend, 1979). Practice contractions near birth give the foetus periodic "squeezes," decreasing oxygen level even further, (Joseph, 1947) while birth itself is so hypoxic that "hypoxia of a certain degree and duration is a normal phenomenon in every delivery," not just in more severe cases. The effects on the foetus due to extreme hypoxia are dramatic: normal foetal breathing stops, foetal heart rate accelerates, then decelerates, and the foetus thrashes about frantically in a life and death struggle to liberate itself from its terrifying asphyxiation (Peter & Peth, 1980). Sometimes continuous seizures (status epilepticus) occur as a result of hypoxia (Lucas, 2002). This causes either focal or global brain damage, with characteristic biochemical and molecular alterations that can result in permanent or transitory neurological sequelae or even death (Rodrigo et al., 2005).

Hypoxia has been implicated in CNS pathology in a number of disorders including stroke, head trauma, neoplasia, vascular malformations and neurodegenerative diseases. Hypoxia in newborn infants results in severe lifelong consequences. The brain, lungs, heart and kidneys are particularly sensitive to low oxygenation (Li & Jackson, 2002). Brain cells are extremely sensitive to oxygen deprivation and begin to die within five minutes after oxygen supply has been blocked. Cerebral cortex is comprised of layers of neurons exhibiting distinct morphologies and synaptic connections (McConnell, 1991). It is a standard practice to resuscitate newborn infants, both term and premature, who are asphyxiated at birth, with 100% oxygen. In addition, a small number of these newborns will require the administration of epinephrine (10μg/kg) and intravenous fluids, which include 10% glucose (500mg/kg body wt) as part of their initial resuscitation. The safety in the use of 100% oxygen in resuscitation has been raised in several studies. Brain damage due to an episode of cerebral hypoxia remains a major problem in the human infant.
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(Tuor et al., 1996). Every year thousands of newborn infants require some form of resuscitation immediately after birth. Over the past decades, neonatal resuscitation programme have been well developed, but some of the procedures employed in these programme are not based on scientific evidence (Nong et al., 2000).

In some animals, splanchnic innervation is immature at birth; yet adrenal catecholamine secretion has been shown to occur during physiological stresses, such as hypoxia (Roger, 2004). Hedner et al., (1980) reported that hypoxia causes an increased catecholamine synthesis in the immature rat adrenal gland, presumably mediated by an increased tyrosine hydroxylase activity induced via splanchnic nerves during hypoxia. Circulating catecholamines originate to a large extent from the adrenal medulla and serve vital functions in relation with cardiovascular, respiratory and metabolic responses to the stress associated with birth (Lagercrantz, 1996). The biological actions of the catecholamines are initiated through their interaction with two different types of specific cell membrane receptors, α- and β-adrenergic receptors. Epinephrine has both α- and β-adrenergic stimulating properties; of which α-adrenergic mediated vasoconstriction is the most important action (Melanie et al., 2002). Vasoconstriction elevates the perfusion pressure during chest compression, enhancing delivery of oxygen to the heart and brain (Berkowitz et al., 1991). α2-adrenoceptors mediate regulatory influence over various physiological, behavioural and endocrine functions and are implicated in conditions such as hypertension, anxiety, endogenous depression and cognitive functions (Maze & Tranquilli, 1991). Stress applied to laboratory animals' results in a decreased density of α2-adrenergic autoreceptors in the hippocampus and amygdala, reflecting down-regulation in response to elevated circulating endogenous catecholamines (Rusnak et al., 1998).

Investigations on the CNS responses to oxygen deprivation are of obvious importance in revealing mechanisms that participate in coordinated behavior of
respiratory and vasomotor responses to hypoxia. Adaptation to continued moderate hypoxia in the rat brain includes structural and metabolic changes. Brain injury in newborns can cause deficits in motor and sensory function (Frances et al., 2001). A large amount of investigation has focused on cytokine and hypoxia-ischemia-mediated injury to the developing cortex and periventricular white matter as the cause of the neurodevelopmental handicaps suffered by infants who have experienced perinatal brain injury. Energy failure, free radical, cytokine and excitatory amino acid release, and caspase-dependant cell death are known to contribute to injury in the neo-cortex, striatum and periventricular white matter (Back et al., 1998; Cheng et al., 1998). However, the degeneration of thalamus and other non-forebrain structures after hypoxia-ischemia is studied less frequently. Injury to somatosensory thalamus has been described in human newborns after hypoxic-ischemia (Barkovich, 1995; Roland et al., 1998) and contribute to sensory motor deficits in infants with perinatal brain injury and cerebral palsy. Damage to the brain during development affects typical patterns of neuronal connectivity (Finlay et al., 1979). The foetal brain can protect itself from hypoxia by increasing cerebral blood flow for a period between one and three hours, but as the brain becomes increasingly acidotic, the blood pressure falls, inducing ischemic injury. Apoptosis, which involves activation of genetically determined cell-suicide programme, has been observed in postmortem brain tissue from infants after hypoxic-ischemic insults (Pulera et al., 1998, Yue et al., 1997). Comparison of adult and immature animal models of hypoxic-ischemia suggests that apoptosis is more prevalent in the immature brain (McDonald et al., 1997, Li et al., 1998). Nakajima et al., (2000) reported that the relative numbers of apoptotic versus necrotic cells in a rodent model of hypoxic ischemia indicate that many regions such as the cerebral cortex and basal ganglia contain high numbers of apoptotic cells for over 7 days after hypoxia-ischemia.
Excitotoxicity refers to death of neurons and certain other cells mediated by excessive stimulation of extracellular excitatory amino acid receptors mainly glutamate (Choi & Rothman, 1990). Glutamate dehydrogenase (GDH) catalyses the reductive interconversion of α-ketoglutarate and L-glutamic acid. Glutamate is a putative neurotransmitter and a precursor of the inhibitory gamma-aminobutyric acid (GABA). It is reported that GDH plays an important regulatory role of glutamate pathway in brain neural network disturbances and neuronal degeneration (Biju & Paulose, 1998). Selected neuronal circuits as well as certain populations of glia such as immature periventricular oligodendroglia die from excitotoxicity triggered by hypoxic ischemia. These patterns of neuropathologic vulnerability are associated with clinical syndromes of neurologic disability such as the extrapyramidal and spastic diplegia forms of cerebral palsy. The cascade of biochemical and histopathologic events triggered by hypoxic ischemia can extend for days to weeks after the insult is triggered, creating the potential for therapeutic interventions (Michael et al., 2001). Normally these receptors mediate physiologic excitatory effects of the dicarboxylic acid glutamate, one of the most ubiquitous and versatile neurotransmitters in the brain. When excessively stimulated by combinations of elevated synaptic levels of glutamate and membrane depolarization associated with ischemia, channels associated with these receptors allow a lethal flow of Ca\(^{2+}\) and sodium to enter neurons. Excitotoxicity appears to be even more intimately involved in the pathogenesis of cell destruction from hypoxic ischemia in the developing brain than in the adult. Although we focus here on neuronal systems, recent evidence suggests that immature white matter can also be damaged by excitotoxicity triggered through glutamate receptors by hypoxic-ischemia (Follett et al., 2000, Gressens, 1999). Developmental differences in the function and expression of glutamate receptors dictated the response of the newborn brain to injury (Deng et al., 2004). N-methyl-D-aspartic acid (NMDA) activity in the
neonatal rat brain is required for cell survival and the factors that regulate apoptosis in
the neonatal brain that play important roles in the final development of the
somatosensory cortex (Anand & Scalzo, 2000).

Cerebral palsy (CP) means "brain paralysis" refers to motor or postural
abnormalities that are noted during early development. These anomalies are thought to
be associated with prenatal, perinatal or postnatal events of varying etiologies (often
multifactorial in nature). CP generally is considered to be a static encephalopathy that
is nonprogressive in nature. The immature brain has only a limited number of ways of
responding to acute or chronic injury and these essentially consist of neuronal and
white matter loss and glial proliferation. These changes occur over many days and
weeks. By the time a child presents with cerebral palsy during the first years of life,
the neuropathological effects of any hypoxic-ischemic injury or other injury will have
become modified by these changes and by further postnatal brain development (Blair
& Stanley, 1988). During the perinatal period and infancy (first 2 years post natal),
there are several incidences that can cause brain damage. Complications with the
endocrine system due to hypoxia include respiratory distress syndrome, hypoglycemia
or hypothyroidism (Nelson & Ellenberg, 1986). Nevertheless CP is secondary to
prenatal, perinatal or neonatal insult; or is secondary to neuronal damage at the
cellular level in the neurotransmitter or receptor systems. The global effects are the
result of impaired communication between the brain and the muscles which decreased
the control of movements that causes poor motor coordination, balance and abnormal
movements. As a result, these motor difficulties are secondary to brain damage or
abnormal brain development. In the individual who has cerebral palsy and epilepsy,
this disruption may be spread throughout the brain and cause varied symptoms all
over the body as in tonic-clonic seizures, or is confined to just one part of the brain
and cause more specific symptoms, as in partial seizures. Neonatal and infantile
seizures suggest underlying structural brain disease with the possibility of adverse motor consequences (Singhi et al., 2003).

Acetylcholine (ACh) is one of the principal neurotransmitters of the parasympathetic system. Extensive evidence supports the view that cholinergic mechanisms modulate learning and memory formation. Evidence for cholinergic regulation of multiple memory systems, noting that manipulations of cholinergic functions in many neural systems can enhance or impair memory for tasks generally associated with those neural systems (Paul, 2003). The magnitude of ACh release in different neural systems regulates the relative contributions of these systems to learning. ACh is the neurotransmitter that is released by stimulation of the vagus nerve, which alters heart muscle contractions. It is important for the movement of other muscles as well. The cholinergic innervation of the cerebral cortex has been extensively investigated because of its role in arousal, learning and memory (Metherate et al., 1992, Voytko et al., 1994). Multiple neuropsychological tests have revealed neuropsychological dysfunction, which is largely due to brain hypoxia. Glucose acts directly on the brain to regulate neural processing, a function that seems incompatible with the traditional view that brain glucose levels are high and invariant except under extreme conditions. However, recent data suggest that the glucose levels of the brain extracellular fluid are lower and more variable than previously supposed (Oltmanns et al., 2004).

In the present work, the role of oxygen, epinephrine and glucose supplementation in regulating neurotransmitter contents, adrenergic and glutamate receptor binding parameters in the cerebral cortex of experimental groups of neonatal rats were investigated. The study of neurotransmitters and their receptors in the cerebral cortex and the EEG pattern in the brain regions of neonatal rats were taken as index for brain damage due to hypoxia, oxygen and epinephrine. Real-Time PCR
work was done to confirm the binding parameters. Second messenger, cyclic Adenosine Monophosphate (cAMP) was assayed to find the functional correlation of the receptors. Behavioural studies were carried out to confirm the biochemical and molecular studies. The efficient and timely supplementation of glucose plays a crucial role in correcting the molecular changes due to hypoxia, oxygen and epinephrine. The addictive neuronal damage effect due to oxygen and epinephrine treatment is another important observation. The corrective measures from the molecular study brought to practice will lead to maintain healthy intellectual capacity during the later developmental stages, which has immense clinical significance in neonatal care.
OBJECTIVES OF THE PRESENT STUDY:

1. To induce hypoxia and supplement glucose, epinephrine and oxygen in the neonatal rats.

2. To measure the blood glucose level in the serum of experimental groups of neonatal rats.

3. To measure the neurotransmitters content in the cerebral cortex and adrenals of the experimental groups of neonatal rats using High Performance Liquid Chromatography.

4. To study the glutamate dehydrogenase activity in the cerebral cortex and liver of experimental groups of neonatal rats.

5. To study the cholinergic activity using acetylcholine esterase in the cerebral cortex and muscle of experimental groups of neonatal rats.

6. To study the adrenergic receptor, $\alpha_2$-AR, $\beta$-AR and glutamate receptor binding parameters in the cerebral cortex of experimental groups of neonatal rats.

7. To study the gene expression of adrenergic receptors - $\alpha_2$A-AR, $\beta_2$-AR and glutamate receptor (NMDAR1) in the cerebral cortex of experimental groups of neonatal rats using Real-Time PCR.
8. To study the second messenger cAMP content in the cerebral cortex of experimental groups of neonatal rats.

9. To study the brain activity generated in the brain regions- Frontal, Temporal, Occipital and Parietal lobes of the experimental groups of neonatal rats using electroencephalogram.

10. To study the behavioural activities of the experimental groups of neonatal rats using Elevated Plus-Maze and Open-Field Test.