**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, 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 lack of motor coordination. The varying levels of functional damage can be reversed 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 degeneration of blood vessels 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). Birth itself is so hypoxic that "hypoxia of a certain degree and duration is a normal phenomenon in every delivery," and not just in 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 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). Brain damage due to an episode of cerebral hypoxia remains a major problem in the human infant (Tuor et al., 1996). Every year thousands of newborn infants require some form of resuscitation immediately after birth. 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. Over the past decades, neonatal resuscitation programmes have been well developed, but some of the procedures employed in these programmes are not based on scientific evidence (Nong et al., 2000).

Dopamine (DA), a major neurotransmitter in central nervous system is involved in the control of motor and cognitive programmes. Dopaminergic neurons
appear early during development (6-8 weeks) in humans. The dopamine turnover is relatively high during perinatal period compared to adults (Herlenius & Lagercrantz, 2001). DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. DA interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-O-methyltransferase (COMT). DA plays an important role both centrally and peripherally. The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. DA receptors are classified into two major groups: DA D₁ like and DA D₂ like. DA D₁ like receptors consists of DA D₁ and DA D₅ receptors. DA D₂ like receptors consists of DA D₂, DA D₃ and DA D₄ receptors. Stimulation of the DA D₁ receptor gives rise to increased production of cAMP. DA D₂ receptors inhibit cAMP production, but activate the inositol phosphate second messenger system (Seeman, 1980). Disturbances of the development of the dopaminergic system lead to dyskinesia, dystonia, tics and abnormal eye movements. An imbalance between dopaminergic neurotransmission and DA receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders, like schizophrenia, psychosis, mania and depression as well as neuropathological disorders, like Parkinson's disease and Huntington's disease (Carlsson, 1988, 1993; Bermanzohn et al., 1992; Brown & Gershon, 1993; Jakel & Maragos, 2000; Kostrzewa & Segura-Aguilar, 2003). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan et al., 1998). During postnatal development, extensive changes takes place in neurotransmitter systems including glutamate, GABA, serotonin, dopamine and acetylcholine in the cortex of primates (Weickert et al., 2007). Of these neurotransmitter systems, dopamine is of particular interest in relation to the development of cognitive abilities subserved by the prefrontal cortex. The most
postsynaptic markers of the DA system are its receptors. Studies done by Gurevich et al., (2000) showed that DA D2 mRNA was found to be expressed in foetal human temporal cortex in differentiated neurons of the cortical plate and cortical sub-plate. Studies on piglets have also shown that the use of dopaminergic receptor antagonists in neonates protects the striatum without the adverse effects of completely blocking the NMDA receptors in the developing brain. The effects of DA D2 receptor actions in the cortex have to be studied in detail.

Investigations on the CNS responses to oxygen deprivation are of obvious importance in revealing mechanisms that participate in coordinated behaviour 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-depandent 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. Injuries to somatosensory thalamus have 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
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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.

Cerebral palsy (CP) meaning "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. 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), several incidences 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 cause poor motor coordination, balance and abnormal movements. As a result, these motor difficulties are secondary to brain damage or abnormal brain development. In individuals with CP and epilepsy, this disruption is 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). 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 glucose, oxygen and epinephrine supplementation in regulating neurotransmitter contents, dopaminergic binding parameters in the brain regions of experimental groups of neonatal rats were investigated. The study of neurotransmitters and their receptors in the cerebral cortex and the Ca^2+ release patterns in 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 messengers - cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP) and inositol 1, 4, 5-trisphosphate (IP3) were 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 sequence of glucose, epinephrine and oxygen administration at the molecular level is an important aspect of the study. The additive neuronal damage effect due to oxygen and epinephrine treatment is another important observation. The corrective measures by initial supply of glucose to hypoxic neonatal rats showed from the molecular study when brought to practice will lead to 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 Wistar neonatal rats.

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

3. To measure the dopamine (DA) and homovanillic acid (HVA) in the brain regions - BS and CB and serum of the experimental groups of neonatal rats using HPLC.

4. To study DA, DA D1, DA D2 receptors binding parameters in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats.

5. To study DA D1 and DA D2 receptor gene expression in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats using Real-Time PCR.

6. To study NMDA receptor binding parameters in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats.

7. To study mGLU5 and NMDA 2b receptor gene expression in the brain regions - CC, BS and CB of experimental groups of Wistar neonatal rats using Real-Time PCR.

8. To study the second messengers - cAMP, cGMP and IP3 content in the brain regions of experimental groups of Wistar neonatal rats.
9. To study the behavioural activities of the experimental groups of Wistar neonatal rats using Rotarod test.

10. To study the Ca\(^{2+}\) patterns in the cortical cells of neonatal rats \textit{in vitro} in confocal microscope.

11. To study the apoptotic pattern in the cortical cells using TO-PRO-3 staining using confocal microscope.