**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). Hypoxic–ischemic (HI) injury in term neonates, whether resulting from birth asphyxia, cardiac arrest or respiratory failure, is known to produce reduced brain growth and associated cognitive, motor and behavioural deficits later in life. The ventilatory response to hypoxia represents the net result of two counteracting stimuli acting upon the central nervous system: 1) an augmentation of neuronal activities through afferent influences from the peripheral chemoreceptors and 2) a reduction of these activities through influences of hypoxia upon the central nervous system (Neubauer *et al.*, 1990; St. John, 1981; Rigatto *et al.*, 1988). If a hypoxic insult occurs during a critical cellular or tissue differentiation process, that episode might have a serious impact on brain maturation. For this reason alone the perinatal age is of great importance, but the process of delivery and the sudden adaptation to postnatal life are in their own right stressful and demanding events for the metabolic homeostasis of the newborn organism. Add on the event of an HI episode to this already stressful time and the results can be catastrophic at best or fatal at worst. 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. 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,
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).

The ventilatory response to acute hypoxia (hypoxic ventilatory response; HVR) in humans and some other mammalian species is biphasic (Neubauer et al., 1990; Weil & Zwillich, 1976). The initial rise in ventilation (early phase of the HVR) is followed by a marked decline after several minutes to values above the prehypoxic level. This decline in ventilation has been termed "ventilatory roll-off" or "hypoxic ventilatory decline" (HVD). Several neurotransmitters and neuromodulators, such as γ-aminobutyric acid (GABA), (Kazemi & Hoop, 1991; Kneussl et al., 1986; Richter et al., 1999; Taveira da Silva et al., 1987) serotonin (5-HT), (Di Pasquale et al., 1992) adenosine, (Elnazir et al., 1996; Neylon & Marshall, 1991) and platelet-derived growth factor (PDGF-β) (Gozal et al., 2000; Simakajornboon & Kuptanon, 2005) play important roles in HVD. Endogenous
GABA acting on GABA$_A$ or GABA$_B$ receptors modulates ventilation during room air breathing as well that the ventilatory response to acute and sustained hypoxia (Zhang et al., 2002). Huang et al (1994) reported that the decrease in ventilation during the biphasic ventilatory response to hypoxia in the neonatal piglet is in part mediated through the effect of GABA on the central nervous system. Long-term hypoxia produces a significant but reversible reduction on GABA binding to GABA$_A$ receptor sites in cerebral cortex, which reflect an adaptive response to this sustained pathophysiological state (Viapiano et al., 2001). Hypoxia has been a selective pressure in conserving GABA and glutamate as major inhibitory and excitatory neurotransmitters in vertebrates as well as invertebrates (Nilsson & Lutz, 1993). GABA$_B$ receptors contribute essentially to the modulation of respiratory rhythm in adult mammals and may be involved in the control of respiratory neuronal discharge (Ai-Lun Yang et al., 2007).

Serotonin (5-hydroxytryptamine; 5-HT) is one of neurotransmitters participating in the development of hypoxia-induced pulmonary hypertension. Pulmonary vasoactive responses to hypoxia are intensified by 5-HT (Eddahibi, 1997). Hypoxia-induced long-term facilitation requires 5-HT receptors and is characteristic of both hypoglossal and phrenic motor output (Bach & Mitchell, 1996; Jacono et al., 2005). 5HT modulates the dynamics of the hypoxic sensory response through its action on 5-HT$_2$ receptors (Serrano et al., 2003). The neurotransmitter acetylcholine (ACh) acting through muscarinic receptors is involved in many aspects of respiratory neuromodulation (Haji et al., 2000), notably central chemosensitivity in brainstem structures (Ballantyne & Scheid, 2000; Burton & Kazemi, 2000) and peripheral chemosensory mechanisms originating in the carotid bodies (Shirahata et al., 2007). Muscarinic receptors are also present on rhythm-generating neurons in the pre-B‘otzinger complex (Shao & Feldman, 2000). The muscarinic receptor stimulation by acetylcholine and 5HT$_2A$ stimulation leads to activation of phospholipase C (PLC), which, in turn, hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce inositol
triphasphate (IP3) and diacylglycerol (DAG) (Best & Malaisse, 1983; Zawalich et al., 1989). G protein coupled receptors like GABA\(_{\beta}\), 5-HT\(_2\) and muscarinic receptors act through second messengers like IP3, cAMP or cGMP. Studying the level of these messengers reveals the control of signaling cascades in the cell.

cAMP responsive element binding protein (CREB) is a protein that is a transcription factor. It binds to certain DNA sequences called cAMP response elements and thereby increases or decreases the transcription of the downstream genes (Lauren, 2005). In neuronal tissue, CREB regulation by nerve growth factor and insulin-like growth factor-1 is essential for neuronal plasticity, full axonal development, memory consolidation and neuroprotection (Spaulding, 1993; Shimomura et al., 1998). The PLC activity decline in the brain is expected to affect DAG which is the principal molecular species of phosphoinositides in the nervous tissue (Whiting et al., 1979). Alterations in glucose utilisation are known to occur in the important regions of brain connected with learning and memory (Auer & Siesjo, 1993).

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. As a result of deprivation of oxygen (hypoxia) and nutrients, the growth and viability of cells is reduced. Hypoxia-inducible factor 1 alpha (HIF-1A) helps to restore oxygen homeostasis by inducing glycolysis, erythropoiesis and angiogenesis (Paul et al., 2004). HIF-1\(\alpha\) protein level is an indicator for hypoxic regions undergoing apoptotic cell death. After exposed to hypoxia, many kinds of cells increase their synthesis of HIF protein, which in turn binds to and activates many genes (Wang et al., 2007).

Perinatal hypoxic ischemic injury is accompanied by neurodegeneration, including features of both necrotic and apoptotic neuronal death as well as destruction of neurites connecting different neuronal populations (Ferriero, 2004).
Apoptosis is regulated and executed by many proteins, which are expressed *de novo* or activated in response to apoptotic signals. The mitochondrial pathway has frequently been implicated in neuronal apoptosis, along with the pro-apoptotic BAX protein, a major component of this pathway. BAX plays a role in cell death following hypoxia and ischemia. The expression of BAX in brain regions can be taken as an index of the brain damage caused by hypoxic stress. The occurrence of hypoxic brain injury during foetal or neonatal development leads to damaged immature neurons and result in behavioural and/or cognitive dysfunction, including motor or learning disabilities, cerebral palsy, epilepsy or even death (Delivoria-Papadopoulos & Mishra, 2000; Levison *et al.*, 2001; Saikumar *et al.*, 1998). Mild hypoxia–ischemia induces significant cerebral injury in neonates and is frequently accompanied by motor and cognitive impairments throughout life (Lindstrom *et al.*, 2006; van Handel *et al.*, 2007). Neither the mechanisms determining the severity of long-term consequences, nor treatment options are sufficiently understood. Perinatal hypoxic–ischemic injury is a serious problem in both full-term and premature human neonates, with a high risk of future behavioural and neurological deficits. In spite of improvements in obstetric and neonatal intensive care, hypoxic–ischemic brain damage with severe neurological disability remains a clinical problem and studies in animal models continue to be of high demand.

Brain cells are extremely sensitive to oxygen deprivation and begin to die within five minutes after oxygen supply has been blocked. 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). 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). Hypoxia in newborn infants is becoming much easier to prevent, detect and treat. Nevertheless the successful management of potentially hypoxic fetuses and newborn infants remains the major challenge to all physicians concerned with perinatal care. What is at stake is not only that sick infants should survive, but equally or more importantly that the survivors should be normal children.

The present study was designed to investigate the protective effect of glucose, oxygen and epinephrine resuscitation on impairment in the functional role of GABAergic, serotonergic, muscarinic receptors, PLC, BAX, SOD, CAT and GPx expression in the brain regions of hypoxia induced neonatal rats. Also, the role of hormones - Triiodothyronine (T3) and insulin, second messengers – cAMP, cGMP and IP3 and transcription factors – HIF and CREB in the regulation of neonatal hypoxia and its resuscitation methods were studied. Behavioural studies were conducted to evaluate the motor function and cognitive deficit in one month old control and experimental rats. 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 in Wistar neonatal rats and resuscitate with glucose, oxygen and epinephrine.

2. To measure the free radical scavenging capability in the heart and cerebral cortex of control, hypoxic and resuscitated groups of neonatal rats by assaying SOD and catalase activity and gene expression of SOD and GPx.

3. To measure the circulating triiodothyronine and insulin level and triiodothyronine receptor binding parameters and insulin receptor expression in control, hypoxic and resuscitated groups of neonatal rats.

4. To measure 5-HT and 5-hydroxy indole acetic acid (5-HIAA) content in the brain regions - BS and CB of control, hypoxic and resuscitated groups of neonatal rats using HPLC.

5. To measure GABA content in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats using displacement method.

6. To study the total GABA, GABA$_A$ and GABA$_B$ receptor subtypes binding parameters in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.

7. To study the total 5-HT and 5-HT$_{2A}$ receptor subtype binding parameters in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.
8. To study the total muscarinic receptor binding parameters in cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.

9. To study the gene expression of GABA$_{A_1}$, GABA$_{A_5}$, GABA$_{A_3}$, GABA$_{A_6}$, GABA$_B$, 5-HT$_2A$, muscarinic M1, muscarinic M2, muscarinic M3 receptors, GAD, 5-HTT, acetylcholine esterase, choline acetyltransferase, transcription factors - HIF1A and CREB, pro- apoptotic protein BAX and enzyme PLC in the cerebral cortex, cerebellum, brain stem and corpus striatum of control, hypoxic and resuscitated groups of neonatal rats using Real Time PCR.

10. To study localisation and expression status of GABA$_{A_1}$, 5-HT and 5-HTT in the brain slices of cerebral cortex, cerebellum and brain stem of control, hypoxic and resuscitated groups of neonatal rats using specific antibodies in confocal microscope.

11. To study the second messengers – cAMP, cGMP and IP3 content in the corpus striatum of control, hypoxic and resuscitated groups of neonatal rats.

12. To study the behavioural changes in control and experimental neonatal rats using Y-maze, radial arm maze, water maze, wire maneuver test and righting reflex.