Discussion

Respiration proves to be a highly integrated process that involves a complex network of interplay between the brain, brain stem, spinal cord, cranial and spinal nerves, diaphragm, intercostal muscles, laryngeal and pharyngeal structures, lungs, and the vasculature. It also involves diverse sets of neurotransmitters, neuromodulators, receptors, second messengers and transcription factors. Hypoxic injury induces early changes in cerebral energy that later lead to the presence and extension of brain damage and subsequently to severe neurodevelopmental impairments. Kranjc et al (1994) reported that maximal neurotransmitter changes occurred 15 minutes after the hypoxic insult. Vulnerability of the neonatal rat brain to hypoxia/ischemia appears to peak at the end of the first postnatal week and then progressively diminish (Ikonomidou et al., 1989). Hyperoxia triggers diffused 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 et al., 2007). Hypoxia induces catecholamine secretion by 2-6 days after birth and initiates the chemoreceptor response as well as nerve activity to hypoxia (Donnelly & Doyle, 1994).

The end of second postnatal week is a highly plastic narrow window of respiratory development. This time window is regarded as the ‘critical period’ previously described as a period ‘devoted to structural and/or functional shaping of the neural system subserving respiratory control’ (Carroll, 2003). This window also marks other bodily changes, such as the opening of eyelids, the opening of the external auditory canal, the onset of non-REM sleep, the onset of the power-law distribution of wake bout distribution, the thickening of fur, a switch from polynervous to mononervous innervation of muscle fibres, the pruning of synapses onto Purkinje cells of the cerebellum and a change from crawling to walking (Jouvet-Mounier et al., 1970; Brown et al., 1976; Crepel et al., 1976;
Hoath, 1986; Petrosini et al., 1990; Blumberg et al., 2005). Other neurochemical and hormonal changes also contribute to dynamic homeostatic regulation at this time. If such a critical period exists in humans (weeks or months in humans instead of days in rats) and if respiratory insults are introduced at this time to a vulnerable infant, especially during sleep when the respiratory control system is further suppressed (Olson & Simon, 1996; Moss, 2002), it is possible that catastrophic events, such as Sudden Infant Death Syndrome, may result. The response to acute hypoxia is indeed biphasic, especially from P3 onward, with ventilation in the first 30 s to 1 min being higher than the rest of the 5-min period. Significantly, this biphasic response undergoes developmental changes during the first 3 postnatal weeks. From P0 to P11, the ratio of minute ventilation (VE) in hypoxia to that in normoxia throughout the 5-min period is >1, indicating that the system is able to respond adequately to hypoxia. However, between P12 and P16, much of the late phase of HVR is <1, suggesting that the depressive effect of hypoxia is much more prominent at this time, despite the fact that ventilation during normoxia is quite high (Liu et al., 2006). This reduction is due mainly to decreased frequency response, but on P13, when HVR is at its lowest, the decrease is caused by a distinct suppression of both frequency and tidal volume (VT). P13 is the only time during development when the VT (hypoxia): VT (normoxia) ratio falls below 1. The marked reduction in HVR at the end of the second postnatal week is consistent with a predominance of inhibitory neurotransmitter expression, a switch in GABA<sub>A</sub> receptor subunits from neonatal to an adult form, as well as heightened expression of GluR2 receptors occurring at the same time in multiple brain stem respiratory nuclei (Wong-Riley & Liu, 2005), all of which point to a stronger inhibitory suppression that dampen the hypoxic ventilatory response. Hence, in our experiments the neonatal rats were subjected to single episode of hypoxia for 30 minutes and sacrificed on day 13 after birth.

Prolonged hypoxemia during infancy is frequently the result of a congenital cyanotic heart defect or lung disease resulting from pre-maturity. A
constant supply of oxygen is indispensable for cardiac viability and function. However, the role of oxygen and oxygen-associated processes in the heart is complex, and they can be either beneficial or contribute to cardiac dysfunction and death. As oxygen is a major determinant of cardiac gene expression and a critical participant in the formation of ROS and numerous other cellular processes, consideration of its role in the heart is essential in understanding the pathogenesis of cardiac dysfunction (Giordano, 2005). As myocardial O\textsubscript{2} levels decrease, either during isolated hypoxia or ischemia-associated hypoxia, gene expression patterns in the heart are significantly altered (Huang et al., 2004). Prior studies have revealed that respiratory as well as cardiovascular function and control at maturity were abnormal after hypoxemia experienced in early life (Sorensen & Severinghaus., 1968; Okubo & Mortola., 1990., Rohlicek et al., 2002). Considering the impact of hypoxia on cardiac tissue, we evaluated the free radical scavenging system, insulin and T3 receptor expression in the heart of experimental groups of rats. The dysfunction of antioxidant and hormonal systems in the heart under neonatal hypoxia further points to the need for devising an immediate and proper resuscitation method to overcome the stress.

GABA, the principal inhibitory neurotransmitter in the CNS and peripheral nervous system (PNS), maintains the inhibitory tones that counter balances neuronal excitation. Glutamate decarboxylase enzyme (GAD) is the rate limiting enzyme of GABA synthesis and it is used as a marker for GABAergic activity. Glucose regulatory control is mediated by the interplay between inhibitory GABAergic neurotransmission and excitatory glutamatergic neurotransmission within the CNS. Serotonin or 5-Hydroxytryptamine (5-HT) is a monoamine neurotransmitter, which act through serotonin receptors. It influences various biological and neurological processes such as aggression, anxiety, appetite, cognition, learning, memory, mood, nausea, sleep and thermoregulation. 5-HT transporter (5-HTT) is an integral membrane protein that transports serotonin from synaptic spaces into presynaptic neurons. This transport of serotonin by the 5-HTT
terminates the action of serotonin and recycles it in a sodium-dependent manner. Acetylcholine, a major neurotransmitter in the autonomic nervous system plays an integral role in normal muscle functions, motor activity, attention, fear, anxiety and learning through interactions with muscarinic and nicotinic receptors. Acetylcholine level in synapse is regulated by choline acetyltransferase, the acetylcholine synthesizing enzyme and acetylcholine esterase which degrade the acetylcholine. Hypoxia damages the GABAergic, serotonergic and cholinergic functions. Hypoxic insult during neonatal period is associated with an apoptotic cell death in the CNS resulting in behavioural changes in the later stages of life.

Several neurotransmitters and neuromodulators, such as GABA, 5-HT, adenosine and platelet-derived growth factor – β play important roles in hypoxic ventilatory decline (Kazemi & Hoop., 1991; Elnazir et al., 1996). 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). Serotonin 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). Muscarinic receptors are also present on rhythm-generating neurons in the brain stem. The muscarinic receptor stimulation by acetylcholine and 5-HT_2A leads to activation of phospholipase C (PLC), which in turn hydrolyses phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce Inositol triphosphate (IP3) and diacylglycerol (DAG).

The signaling from the neurotransmitters is carried to the cell nucleus by second messengers like cAMP, cGMP and IP3. Their expression and changes play a major role in the signaling cascade. Different transcription factors are modulated during hypoxia in order to overcome the stressful condition. The most important among them is hypoxia inducible factor 1 (HIF 1) and cAMP responsive element binding protein (CREB). HIF-1α plays an essential role in cellular oxygen homeostasis by regulating the expression of genes involved in glycolysis,
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erythropoiesis and angiogenesis. HIF-1α is also a key component of the cellular response to hypoxia and ischemia under pathophysiological conditions. CREB plays an important role in a variety of cellular processes, including proliferation, differentiation and adaptive responses. Increased CREB phosphorylation during post-ischemic recovery is associated with neuronal survival.

Free radical toxicity mediated neurodegeneration and apoptotic cell death are two important factors which contributes to brain damage in hypoxic condition. So an effective resuscitation method should help in enhancing the free radical scavenging capability and reducing the expression of pro-apoptotic genes. The free radical scavenging system consists of enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes were reported to show decreased activity under hypoxic condition. An effective resuscitation programme to encounter neonatal hypoxia should ensure minimum brain damage and free radical formation with a balanced expression of various neurotransmitters and its signaling pathways, so that the harmony of the body functions is not disturbed.

BODY WEIGHT AND BLOOD GLUCOSE LEVEL

There was no significant change in body weight of hypoxic rats when compared to control on postnatal day 13. Behavioural study was conducted on postnatal day 30. The 1 month old hypoxic rats showed a significant decrease in the body weight compared to control. When animals are acutely subjected to hypoxia, food intake declines and hence results in decreased body weight. Golan et al., (2004) reported that hypoxic episode in early life stage caused impairment in the morphogenic parameters and motor strength in the newborns during the first month of age.

Hypoxic insult to four day old neonate did not cause any significant change in the blood glucose compared to control. Supplementation of glucose, oxygen and epinephrine does not cause significant change in blood glucose level
after one week. Maintaining blood glucose level continues to be important throughout the pregnancy, but it is particularly important during early developmental stage, when an embryo's organs are forming. Oxygen is needed by cells to break down glucose and produce energy. The oxygen requirement is more during embryonic development for all the tissues. Insufficient oxygen supply cause developmental abnormalities and birth defects. Thus hypoxia has a potential to cause damage to cells (Rulin et al., 2005). Epinephrine stimulated mechanical performance and heart rate of hypoxic hearts, but decreased myocardial glycogen and ATP. Though glucose utilization remained unchanged, the release of lactate increased from hypoxic hearts treated with epinephrine. However, epinephrine failed to stimulate myocardial lipolysis in hypoxic hearts. These metabolic changes due to epinephrine would lead to accelerated depletion of energetic reserves in hypoxic heart and its earlier deterioration.

FREE RADICAL SCAVENGING IN HYPOXIA.

Oxygen or glucose deprivation alters electrical transmission in the brain and generates free radicals, which mediate neuronal death (Pedersen et al., 1998). Free radical production has been proposed to be involved in the pathogenesis of the ischemia-reperfusion neuronal damage (Globus et al., 1995; Ozben, 1998). Damage to lipids, proteins and nucleic acids has been observed concomitantly with their production, ultimately resulting in cell function impairment and death (Halliwell & Gutteridge, 1990). Reactive oxygen is a signalling molecule and its levels in tissue determine the aging process and lifespan (Sasaki et al., 2010). Increased ventilatory drive following chronic intermittent hypoxia represents a form a neural plasticity which is a ROS dependent phenomenon (Edge et al., 2010)

Free radical scavenging enzymes like SOD, CAT and GPx play important role in protection against oxygen toxicity in mammalian systems (Liu et al., 1977). Studies have demonstrated that free radicals are formed under hypoxic
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conditions in newborn piglet brain (Torres et al., 2004). Hypoxia induces superoxide accumulation in pulmonary artery myocytes through inhibition of mitochondrial SOD activity, promoting peroxynitrite-induced generation of 8-isoprostane (Gong et al., 2010). In the cerebral cortex of rats subjected to 24 hours of reperfusion following 2 h of cerebral ischemia, the SOD activity was decreased (Tsinghua, 2002). The rat endothelial cells produce SOD inhibitable ROS which are augmented by hypoxia/reoxygenation (Strasser et al., 1997). The present study showed that both SOD and CAT activities are significantly less in hypoxic neonatal rats compared to control in the heart and cerebral cortex with a down regulated gene expression of SOD and GPx mRNA, indicating that limitation in oxygen supply is associated with reduction in antioxidant enzymes like SOD, CAT and GPx.

Hyperoxia with 100% oxygen after hypoxic-ischemia cause more damage in the cerebral cortex than room air in newborn rats (Shimabuku, 2005). 100% oxygen generates abnormally high levels of ROS which cause dysfunction of defensive antioxidant system of cells by altering enzyme activity (Bandypadhyay et al., 1999) and act as a factor for neurodegeneration (Matharan et al., 2004). Hypoxemic piglets resuscitated with 100% oxygen also showed increased cerebral injury, cortical damage and early neurological disorders (Shimabuku et al., 2000). In the present study, the increased SOD and CAT activities with decreased affinity observed in hypoxic rats resuscitated with oxygen indicates the decreased function of free radical scavenging enzymes, which add to more damage due to the free radical formation. The gene expression of SOD and GPx showed an up regulation in the brain regions as a homeostatic response to counter act the excess oxygen free radicals formed. This highlights the role of free radicals in causing damage to brain and heart in neonatal hypoxic rats on 100% oxygen resuscitation.

The present study showed that glucose supplementation to hypoxic and hypoxic treated with oxygen has an efficient free radical scavenging capability compared to all other experimental groups. Hypoxic neonates treated with glucose
have shown a higher activity of SOD and CAT, showing an increased antioxidant capability in presence of glucose. The combination of glucose, epinephrine and oxygen as resuscitation in hypoxic condition has shown a decreased SOD and CAT activity, indicating that free radical toxicity is high in heart and cerebral cortex, due to the administration of epinephrine. Reduction in blood glucose levels and substantially increased cerebral glucose utilization was observed as a result of hypoxic stress in experimental rats (Hattori & Wasterlain, 2004; Vannucci & Hagberg, 2004). Unlike the adult, where glucose supplementation prior to or during hypoxic-ischemia accentuates tissue injury, glucose treatment of perinatal animals subjected to a similar insult substantially reduces the extent of tissue injury (Vannucci & Hagberg, 2004). Hypoxia induced expressional and functional changes in NMDAR1 receptors of neuronal cells in neonatal rats are corrected by supplementation of glucose alone or glucose, followed by oxygen during the resuscitation to prevent the glutamate related neuronal damage (Paulose et al., 2007). Post hypoxic glucose supplement also reduces an elevated brain lactate level which is responsible for cerebral infarction occurring during the hypoxia (Hattori & Wasterlain, 2004). The present study reported the protective role of glucose supplementation by ameliorating the free radical mediated toxicity in cerebral cortex and heart.

**INSULIN LEVEL AND INSULIN RECEPTOR**

Hypoxic stress is well known to decrease appetite and weight gain in growing rats and to induce weight loss in humans at high altitude (Tschop & Morrison, 2001; Raff, 2003). It also increases the expression of a variety of genes with products that act in synergy to facilitate the supply of metabolic energy (Yasumasu et al., 2002). Insulin levels are expected to drop with weight loss; however, in the hypoxic group despite weight loss, insulin levels were higher than in the control group. This implies that hypoxia has a direct effect on insulin independent of weight changes. Hypoxia was previously shown to increase insulin
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messenger RNA (Tillmar & Welsh, 2002). Baum (1969), Baum and Porte (1976) were the only group to report the inhibition of insulin release during hypoxia when studying hypoxic puppies. Their results are in contradiction with our study and with most of previously published results (Bitar et al., 1994; Raff et al., 2001; Tillmar & Welsh, 2002; Meissner et al., 2003). Insulin inhibits hypoxia-induced cleavage of apoptotic factor caspase-3, mediated by p38 mitogen-activated protein kinase (MAPK). Insulin-induced MKP-1 expression was mediated by extracellular signal-regulated protein kinases (ERK) 1/2, c-Jun NH2-terminal kinases (JNK), MAPK and phosphatidylinositol 3-kinase (PI3K)/Akt pathways. Concomitant activation of Akt, ERK 1/2 and JNK was required for insulin to exert its protective effect against the hypoxia-induced cleavage of caspase-3 (Morisco et al., 2007). The increased serum insulin level with up regulated insulin receptors in heart observed in the present study is suggested to be an adaptive modification in the body to overcome energy depletion and to prevent apoptotic cell death.

Both hypoxia and insulin induce common target genes, including vascular endothelial growth factors and several glycolytic enzymes. However, these two signals eventually trigger quite different metabolic pathways. Hypoxia induces glycolysis, resulting in anaerobic ATP production, while insulin increases glycolysis for energy storage. Energy stores are maintained during hypoxic pulmonary vasoconstriction and that this is dependent on glucose availability and up regulation of glycolysis (Leach et al., 2002). Severe hypoxia results in ATP depletion to cells and hence to provide immediate energy glucose is used in the resuscitation practice. Insulin enables the body cells to take glucose from the bloodstream. Circulating insulin increased in response to increased glucose content, which is observed in our glucose supplemented groups. The increased insulin helps in metabolising glucose to derive energy to encounter hypoxic stress. To prevent the on set of cardiac ischemia due to hypoxic stress, heart underwent adaptive changes like increased expression of insulin receptors. Qin et al., (2010) postulated that in mice intermittent hypoxia reduces body weight and serum
glucose by increasing erythropoietin synthesis which secondarily increases leptin and insulin production in liver.

In oxygen supplemented group (Hx + O), the circulating insulin was significantly less with a significant down regulation of insulin receptor gene compared to control. It has been reported that during hypoxia, oxygen supplementation results in the free radical formation and glutamate mediated excitotoxicity (Finla et al., 2008). Reactive oxygen species and reactive nitrogen species act as negative regulators of insulin signalling, rendering them putative mediators in the development of insulin resistance, a common endocrine abnormality that accompanies many diseases (Bashan et al., 2009). In epinephrine supplemented groups (Hx + E, Hx + G + E + O), a very high circulating insulin was observed which contribute to insulin resistance. The insulin receptor expression was significantly up regulated in these groups. Insulin resistance in cells reduces glucose uptake thereby increasing the stress during hypoxia. Epinephrine is known to inhibit insulin secretion (Deibert & DeFronzo, 1980) but will also cause hyperglycemia, which can secondarily increase insulin secretion (Eigler et al., 1979). An increase in plasma epinephrine concentration causes insulin resistance (Porte, 1967; Saccè et al., 1979).

**TRIIODOTHYRONINE AND ITS RECEPTOR**

Thyroid hormones are the most significant factors in regulating energy transformations. Thyroid hormones are involved in setting the basal metabolic rate in many target tissues, such as liver, heart, kidney and brain. Corresponding changes in respiratory activity occur in mitochondria isolated from tissues of hypo- and hyperthyroid animals.

The evaluation of T3 content in serum and heart revealed a significant decrease in hypoxic neonatal rats. This may be an adaptive response of the body to encounter the hypoxic insult. Due to decreased T3 level there will be a reduction in carbohydrate, fat and protein metabolism, so that energy can be utilised for
compensating the ATP depletion during hypoxia. Sawhney and Malhotra (1990) reported a decline in thyroid hormone levels and their production in rabbits under hypoxic stress. Simonides et al., (2008) also reported a mechanism of metabolic regulation during hypoxic-ischemic injury in which HIF-1 reduces local thyroid hormone signalling through induction of type 3 deiodinase. The deficiency of the thyroid hormones results in decreased metabolism and lowers the basal metabolic rate. Thyroid deficiency severely impaired the oxidative energy metabolism in rat brain mitochondria which was accompanied by decrease in cytochromes aa3 and cco1 contents and delayed pattern of mitochondrial protein turnover (Rajwade et al., 1975; Katyare et al., 1977). Hypoxia-induced signalling appears to drive type 3 deiodinase expression in the hypertrophic cardiomyocyte. Many cardiac genes are transcriptionally regulated by T3 and impairment of T3 signalling will not only reduce energy turnover, but also lead to changes in gene expression that contribute to contractile dysfunction in pathologic remodelling (Pol et al., 2010).

In glucose supplemented groups, Hx + G and Hx + G + O, an increased T3 content was observed compared to hypoxic group. T3 increases metabolism including carbohydrate utilisation and hence it increases glucose uptake. Thyroid hormone induces an increase in cardiac contractility and frequency, resulting in a greater cardiac output (Polikar et al., 1993; Toft & Boon; 2000) and a proportional change in energy turnover (Clausen et al., 1991). Kvetny et al (1990) showed that glucose feeding increases serum T3 levels, decreases nuclear T3 binding and enhances thyroid hormone stimulated oxygen consumption and glucose uptake. Since glucose administration is reported to have a role in enhancing T3 stimulated oxygen consumption glucose supplementation during hypoxic stress will help to increase the ventilatory response. The supplementation of oxygen alone showed a decreased T3 content in heart and serum. The decreased T3 is an adaptation of the body to increase the oxygen availability of blood. Resuscitation of hypoxic neonates with 100% oxygen was reported to cause free radical mediated injury in heart and brain. T3 induces a state of oxidative stress mainly by stimulating
mitochondrial ROS generation and not by decreasing mitochondrial antioxidant systems. The increase in \( \text{O}_{2}^- \) production observed by T3 is due to the interaction of the hormone with the mitochondrial membrane lipid bilayer leading to alterations in membrane structure and increased electron leakage at the respiratory chain (Castilho et al., 1998). The activation of mitochondrial respiration by 100% oxygen resuscitation to hypoxic rats releases ROS. Since T3 causes ROS production, its level is decreased as an adaptive response to diminish free radical toxicity. Epinephrine administration stimulates lipolysis, which causes energy utilisation by cells. In order to reduce energy need T3 level was decreased in epinephrine supplemented groups as a homeostatic response.

Thyroid hormone dependent stimulation of target genes requires the interaction of thyroid hormone receptors with specific nucleotide sequences called thyroid-hormone-responsive elements. Lowering of available oxygen (hypoxia) caused a decrease in binding capacity of T3 in the cerebral cortex measured in vitro (Thrall, 1983). T3 receptor can recognize specific DNA sequences and suggest that it can act directly as a positive transcriptional regulatory factor (Koenig et al., 1987). In the present study, T3 receptor showed a significant decrease under hypoxic condition. Glucose supplementation showed a reversal of the receptor status to near control. The receptor binding data is in accordance to the T3 level observed in heart.

**CENTRAL GABAERGIC RECEPTOR ALTERATIONS.**

The ventilatory response to hypoxia is influenced by the balance between inhibitory (GABA, glycine, and taurine) and excitatory (glutamate and aspartate) amino acid neurotransmitters. During and after hypoxia, extracellular levels of amino acid neurotransmitters increase in many brain regions (Hagberg et al., 1987), causing an imbalance of excitatory and inhibitory neurotransmission leading to excitotoxicity (Yue et al., 1997). GABA\(_\alpha\) receptor subunit composition determines the affinity for and efficacy of GABA with binding sites at the GABA\(_\alpha\)
receptor (Hevers & Luddens 1998; Whiting et al 1999; Belelli et al., 2002; Jurd et al 2003). $\text{GABA}_A$ receptor subunits exhibit distinct anatomical specificity in the neural regions (Laurie et al 1992a; Wisden et al 1992; Pirker et al 2000), the types of neurons (Gao & Fritschy 1994; Fritschy & Mohler 1995; Schwarzer et al 2001), and the specific parts of neurons in which they are expressed (Nusser et al 1996; Fritschy et al 1998; Brunig et al 2002). Subunit expression exhibits remarkable plasticity across development (Laurie et al 1992b), in response to changes in physiological states (Fenelon & Herbison, 1996), and in response to environmental challenges (Devaud et al 1995; Cullinan & Wolfe 2000). In particular, $\text{GABA}_{\alpha}$ subunits differentially contribute to various physiologic/psychological/behavioral functions mediated by $\text{GABA}_A$ receptors (Rudolph et al., 1999; Low et al., 2000; McKernan et al., 2000; Tauber et al., 2003) and to the cortical plasticity which in turn mediates environmental influence on neuronal function (Fagiolini et al., 2004).

GABA and glutamate are the two important neurotransmitters involved in hypoxic ventilatory response. GABA in the nucleus tractus solitarii has a pivotal role in the hypoxic ventilatory decline (HVD) and this mechanism is not activated without chemoreceptor stimulation (Tabata et al., 2001). Anoxia-tolerant vertebrates decrease their metabolic rate by 70% or more during anoxia, with an increase in concentration of GABA (Nilsson & Lutz, 2004). 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 CONTENT IN BRAIN REGIONS**

GABA is one of the most abundant neurotransmitters in the vertebrate central nervous system and is involved in neuroendocrine processes such as development, reproduction, feeding and stress (Martyniuk et al., 2005). A decrease in GABA content was observed during neonatal hypoxic insult in
cerebral cortex, brain stem, cerebellum and corpus striatum. The decreased content in the brain regions were reversed to basal level in hypoxic neonates resuscitated with glucose. A reduction of GABAergic input onto caudal hypothalamic neurons in spontaneously hypertensive rats was reported earlier (Shonis et al., 1993). Diminished GABAergic inhibition in this region would ultimately translate into an elevated arterial pressure since activation of neurons in the caudal hypothalamus increases sympathetic nerve discharge to several vascular beds and to the heart (Waldrop & Bauer, 1989). Reductions in GABA concentrations and glutamic acid decarboxylase gene expression are consistent with a reduced GABA receptor function in different brain regions. Reduction in GABAergic inhibition in brain regions contribute to stimulation of heart functions and sympathetic innervations to overcome hypoxia. Hypoxia stimulates caudal hypothalamic neurons with a cardiovascular or sympathetic related discharge in both intact and peripherally chemodenervated cats (Dillon & Waldrop, 1993). Blockade of excitatory input onto the caudal hypothalamus was reported to attenuate the respiratory response to hypoxia in the anesthetized rat (Horn & Waldrop, 1994). It is known that hypoxia rapidly suppresses excitatory transmission between glutamatergic terminals and the inhibitory interneurons, leading to the subsequent failure of inhibitory GABAergic transmission to pyramidal cells (Congar et al., 1995).

**Cerebral cortex**

GABA increases total cerebral blood flow, acting on specific receptor sites in the cerebral blood vessels (Alborch et al., 1984). Long-term hypoxia produces a significant but reversible reduction on GABA binding to GABA<sub>A</sub> receptor sites in cerebral cortex, which reflect an adaptive response to this sustained pathophysiological state (Viapiano et al., 2001). Hypothermic newborn piglets have a depressed ventilatory response to hypoxia due to an increase in central nervous system GABA levels (Qiming xiao et al., 2000). Infusion of the
GABA antagonist bicuculline caused augmentation of the hyperventilatory response to acute hypoxia (Homayoun kazem, 2006). GABA<sub>A</sub> receptors mediate the majority of fast inhibitory synaptic interactions in the mammalian brain. In the adult brain, networks of neurons containing GABA<sub>A</sub>ergic receptors have been implicated in the maintenance of rhythmic activities of neuronal circuits (Whittington et al., 1995; Wang & Buzsaki, 1996) and the precise control of the timing of excitability in individual neurons (Tsubokawa & Ross, 1996). GABA neurotransmission serve both excitatory and inhibitory roles during early development (Chen et al., 1996). Subunit diversity appears to underlie distinctive roles for GABA<sub>A</sub> receptors in the developing nervous system (Laurie et al., 1992; Poulter et al., 1992; Ma et al., 1993).

The binding studies of total GABA and its subtypes – GABA<sub>A</sub> and GABA<sub>B</sub> in the cerebral cortex revealed a significant decrease in the receptor number with a decreased affinity in hypoxic neonates. The expression of GAD was significantly down regulated in hypoxic neonatal rats. The data presented here suggests that decreased levels of key proteins in the GABA pathway in the cerebral cortex lead to high susceptibility to seizures and epilepsy in newborns after prenatal hypoxia (Louzoun-Kaplan et al., 2008). The observed decrease in GAD expression in hypoxic neonates significantly decrease GABA level, which is observed by displacement analysis for GABA quantification. Altered cortical GABA neurotransmission contribute to disturbances in diverse functions through affecting the generation of cortical oscillations in conditions like schizophrenia. These oscillatory activities have been proposed to play critical roles in regulating the efficiency of information transfer between neurons and neuronal networks in the cortex (Hashimoto et al., 2010).

Encountering the hypoxic insult by supplementation of glucose alone and glucose along with 100% oxygen showed a reversal in the receptor number and GAD expression to near control in the cerebral cortex. The combination of glucose and oxygen was found to be the most effective resuscitation method. Glucose is
supplemented during hypoxia to provide an immediate resuscitation to the stress condition by acting as an instant source of energy to the brain. Hattori and Wasterlain (2004) observed a reduction in the blood glucose levels and substantially increased cerebral glucose utilization (Vannucci & Hagberg, 2004) as a result of hypoxic stress in experimental rats. We observed that supplementation of glucose is effective in increasing the GABA, GABA_A and GABA_B receptor status in the cortex. Sine glucose provides an immediate and instant energy to tissues it helps in encountering the ATP depletion induced inhibition of respiration. Ito et al (1994) observed a dose-dependent reduction in the cerebral glucose utilization after intravenous administration of various doses of muscimol, an agonist of GABA_A. A linear relationship was observed between the GABA_A receptor occupancy of muscimol and the decrease in the cerebral glucose utilization (Ito et al., 1994). Bailey et al (2007) reported that glucose dose-dependently increased the expression of GABA_A receptor subunits in pancreatic α-cells.

One of the routine methods of resuscitation for severe hypoxia is the immediate administration of oxygen. We observed that 100% oxygen supplementation for neonatal hypoxia is not as effective as the combination of glucose and oxygen or administration of glucose alone. In the cerebral cortex of oxygen supplemented group, total GABA and GABA_B receptors showed a significant decrease compared to control whereas GABA_A receptors showed a decrease below the hypoxic level. Thus, the binding analysis of total GABA, GABA_A and GABA_B showed that administration of 100% oxygen to hypoxic neonates did not bring balance the GABA level to encounter HVD. Hundred percentage of oxygen generated abnormally high levels of reactive oxygen species (ROS) which causes dysfunction of defensive antioxidant system of cells by altering enzyme activity (Bandyopadhyay et al., 1999) and act as a factor for neurodegeneration (Matharan et al, 2004). Hypoxemic piglets resuscitated with 100% O_2 also showed increased cerebral injury, cortical damage and early
neurologic disorders (Temesvari et al., 2001; Munkeby et al., 2004; Shimabuku et al., 2005). Based on behavioural studies and the studies on acetylcholinesterase, Finla et al., (2008) reported the efficiency of glucose and combination of glucose and oxygen resuscitation methods and the damaging effects of oxygen supplementation alone. The reduction in GABA and its subtypes receptor function in the cortex during oxygen supplementation is suggested to be due to tissue damage caused by the formation of free radicals or reactive oxygen species. Oxidative stress potentiate presynaptic GABA release through the mechanism of cAMP-dependent protein kinase A (PKA)-dependent pathways, which result in the inhibition of the cerebral cortex neuronal activity (Hahm et al., 2010).

Epinephrine and GABA acts antagonistically in a biological system. The over stimulation of brain caused by epinephrine innervation is antagonized by the activity of GABA. Resuscitation of hypoxic neonatal rats with epinephrine triggers its receptors and pathways. The stimulatory action of epinephrine causes a feedback regulation of GABAergic pathways thereby decreasing the GABA receptor function in epinephrine resuscitated groups.

Cerebellum

Cerebellum significantly differs with respect to ischemia and hypoxia, this response being directly related to the duration and intensity of the injury. Cerebellar hypoxia is responsible for important aspects of cognitive deterioration and motor disturbances in neurological disorders, such as stroke, vascular dementia and neurodegeneration. The cerebellum plays important role by boosting the nitric oxide synthase activity to cover the eventual need for nitric oxide during hypoxia (Rodrigo et al., 2004). Hypoxia retards the development of neuronal processes, resulting in a smaller cerebellum (Yu & Wan-Hua Yu, 1980).

In the present study, a decreased total GABA, GABA<sub>A</sub> and GABA<sub>B</sub> receptor number with a down regulated GABA<sub>A</sub>α<sub>1</sub>, GABA<sub>A</sub>α<sub>5</sub>, GABA<sub>A</sub>γ<sub>3</sub>, GABA<sub>A</sub>δ and GABA<sub>B</sub> receptor subunits gene expression was observed in the cerebellum of
neonatal rats exposed to hypoxic stress. During hypoxic insult, body tries to encounter the stress by increasing its metabolic rate, thereby resulting in ATP depletion. The high metabolic cost for various cellular adaptations to hypoxia results in a decrease in neuronal activity and synaptic transmission. GABA receptors were decreased as an adaptive response of the body to prevent energy depletion. Hypoxia activates c-Jun NH (2)-terminal kinase (JNK) signaling pathway. Stimulation of GABA receptors attenuate this apoptotic pathway (Han et al., 2008). Since the receptors were decreased during hypoxic stress, the apoptotic pathway will be triggered resulting in brain cell death. An effective resuscitation method should inhibit JNK apoptotic pathway to prevent brain injury.

Hypoxia induced expressional and functional changes in GABA receptors in the cerebellum of neonatal rats are corrected by supplementation of glucose alone or glucose, followed by oxygen during the resuscitation, which prevented the GABA induced ventilatory decline. A significant reversal in receptor number to near control was observed in glucose supplemented groups. The gene expression of GABA subunits also showed a reversal to near control in a differential manner. The reversal of GABA sensit receptors to near control will augment Akt activation and thereby inhibit the JNK cascade. Differential expression of GABA receptor subunits represents a major aspect of homeostatic synaptic plasticity and contributes to the excitation/ inhibition balance under physiological conditions and upon pathological challenges.

Hypoxia is a general factor affecting glucose metabolism. Gillesen and Kammermeier (1999) reported that moderate glucose elevation exerted beneficial effects on hypoxic hearts: the depressed contraction was improved, the action potential shortening partly reversed and the percentage of irreversibly damaged myocytes diminished. Reduction in blood glucose levels and substantially increased cerebral glucose utilization was observed as a result of hypoxic stress in experimental rats (Hattori & Wasterlain, 2004). The neuroprotective effect of
glucose supplementation to hypoxic neonates on dopamine receptor and glutamate receptors was already reported (Joseph et al., 2010).

Resuscitation of hypoxic neonates with 100% oxygen decreased the GABA receptor number and down regulated the gene expression of GABA_A and GABA_B subunits. Hundred percentage of oxygen generated abnormally high levels of reactive oxygen species (ROS) which causes dysfunction of antioxidant system of cells by altering enzyme activity. GABA neurotransmission is sensitive to reactive oxygen species. ROS contributes to GABA_A-mediated neuronal inhibition via interaction with pre and postsynaptic sites. A reduction in GABA_A-gated Cl^- channel function during periods of oxidative stress contribute to the development of neuronal damage (Sah et al., 2000). This resuscitation method was already reported to cause glutamate and dopamine mediated excitotoxicity. Our study points out the adverse effects of epinephrine supplementation, alone and even in combination with glucose and oxygen. The resuscitation attempt using epinephrine cause a hyperactivity of excitatory stimulus, which might have affected the various neurotransmitter levels thereby declining body’s adaptations to overcome hypoxia.

**Brain stem**

Respiratory control centers in the brain stem are located in three main areas: pons, dorsal medulla and ventrolateral medulla (Bianchi et al., 1995; Feldman et al., 2003). GABA and glycine are the two major inhibitory neurotransmitters in the network of respiratory neurons in the brain stem and spinal cord (Haji et al., 2000). GABA, acting mainly on GABA_A receptors and glycine mediate fast synaptic inhibition via chloride channels and help shape the discharge patterns of all respiratory neurons (Bianchi et al., 1995). GABA_B receptors, on the other hand, are slower-acting metabotropic receptors coupled to Ca^{2+} and K^+ channels via G proteins and second messengers (Kerr & Ong, 1995;
Takahashi et al., 1998). They reportedly modulate respiratory rhythm in adult mammals (Gray et al., 1999).

Binding studies using radio labeled ligands showed a significant decrease in total GABA, GABA\textsubscript{A} and GABA\textsubscript{B} receptor number with a down regulated expression of receptor subtypes and GAD in hypoxic neonatal rats. The increased glutamate and 5-HT activation for transmission of inspiratory drive in the respiratory centers of brain stem negatively regulated the GABA receptors. The resuscitation with glucose alone and with oxygen effectively reversed the changed parameters to near control. Resuscitation with epinephrine and oxygen alone did not show any significant reversal of GABAergic function to control. The alterations in GABA receptors under hypoxic stress are due to negative regulation by other neurotransmitters and also a response of ATP depletion. Since glucose supplementation along with oxygenation provides an immediate energy source the imbalance in GABAergic function can be reversed by timely supplementation of glucose.

**Corpus striatum**

The striatum is involved in mediating habit learning (Berke & Hyman, 2000) and optimizing control of motor behavior and cognitive function (Graybiel, 2005) and is susceptible to oxygen deprivation. Approximately 90% of striatal neurons are medium spiny neurons (Calabresi et al., 2003; Baquet et al., 2004), which are selectively targeted during glucose (Calabresi et al., 1995\textsuperscript{a}) and oxygen deprivation (Calabresi et al., 1995\textsuperscript{b}, 2000). GABA is considered as the main transmitter released from the axons of striatal neurons projecting to the output structures of the basal ganglia. It also plays a central role in the processing of information in the striatum (Groves, 1983). The striatum also receives GABAergic afferents from globus pallidus and substantia nigra (pars reticulata) (Kita, 1993). Another important intrastratial source of GABA is represented by an extensive network of axon collaterals originating from spiny neurones (Preston et al., 1979;
Park et al., 1980; Wilson & Groves, 1980; Kita, 1993). Striatal neurons are known to have both GABA_A and GABA_B receptors (Ng & Yung, 2001), activation of which decrease potentiation evoked-glutamate and dopamine release in many brain regions including hippocampus, cortex and spinal cord (Tanaka et al., 2002; Matsumoto et al., 2003; Tanaka et al., 2003). GABA protect neurons not only by directly hyperpolarizing neurons but also by exerting an inhibitory influence on glutamate-mediated neuronal activity (Costa et al., 2004).

Neonatal hypoxic stress was observed to decrease the GABA receptor number – both GABA_A and GABA_B with a down regulated gene expression of its subunits. Under hypoxic condition the striatal neurons innervate the glutameric system to facilitate a respiratory trigger to other regions of brain. This accounts for the decline in GABA receptors in striatum. This in turn leads to hypoxic ventilatory decline and glutamate mediated excitotoxicity in hypoxic neonatal rats. Since glucose resuscitated groups reversed the altered GABA receptor function to near control it was suggested to be an effective method to prevent ventilatory decline and neurotoxicity mediated through glutamate excitation. Immediate resuscitation with 100% oxygen and administration of epinephrine resulted in a sudden change of the cellular environment from anaerobic to aerobic. This resulted in the formation of more reactive oxygen species and glutamergic innervation, which in turn leads to decreased GABA receptors and ventilatory roll off. This points to the fact that 100% oxygen or epinephrine resuscitation to hypoxic neonatal rats was not able to reverse the disturbances and alterations in the molecular level due to hypoxic stress.

**DIFFERENTIAL EXPRESSION OF GABA_A RECEPTORS IN BRAIN REGIONS**

GABA_A receptors are composed of five protein subunits that belong to different subunit classes. There are 19 genes for GABA_A-R subunits (Simon et al., 2004). These include 16 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π) combined as
GABA$\alpha$, and 3 rho ($\rho$) subunits which contribute to what have sometimes been called GABA$\gamma$ receptors. The assembly of GABA$\alpha$-R as heteropentamers produces complex heterogeneity in their structure, which is the major determinant of their pharmacological profile (Barnard et al., 1998; Olsen & Sieghart, 2008). The various receptor subtypes differ in abundance in cells throughout the nervous system and thus in functions related to the circuits involved. An important criterion for association of subunit isoforms into oligomeric native receptors is co-localization of the subunits. Immunocytochemical studies investigating the co-localization of subunits in GABA$\alpha$ receptor clusters on neuronal membranes (Fritschy et al., 1992; Bohlhalter et al., 1996), as well as electron microscopic studies (Nusser et al., 1995; Somogyi et al., 1996), indicate that the majority of GABA$\alpha$ receptors present in the brain are composed of $\alpha$, $\beta$, and $\gamma$ subunits.

We observed a differential regulation of GABA$\alpha$ receptor subunits in different brain regions under neonatal hypoxic insult and its resuscitation conditions. In cerebellum, brain stem and corpus striatum GABA$\alpha$1, GABA$\alpha$5, GABA$\gamma$3 and GABA$\delta$ receptor expression was down regulated under hypoxic stress. In cerebral cortex GABA$\alpha$1, GABA$\alpha$5 and GABA$\gamma$3 receptor expression was down regulated with an up regulated GABA$\delta$ expression. The up regulation of GABA$\delta$ receptor subunit in cortex is an adaptive modification to increase conductance and tonic inhibition. Receptors containing $\delta$ subunits exhibit a smaller single channel conductance and a much longer open time and do not desensitize on the prolonged presence of GABA (Saxena & Macdonald, 1994). Together with the exclusive extrasynaptic localization of these receptors, these properties indicate that tonic inhibition observed in these cells is mediated mainly by the persistent activation of $\alpha6\beta3\delta$ receptors by GABA that is present in the extracellular space of glomeruli (Nusser et al., 1998; Brickley et al., 1999).

In glucose resuscitated groups, the GABA$\alpha$1, GABA$\alpha$5, GABA$\gamma$3 and GABA$\delta$ receptor subunits expression was reversed to near control in all the brain regions. When hypoxic neonatal rats were resuscitated with 100% oxygen alone, a
reversal in subunit receptor expression was evident only in the cerebellum, a region which is highly sensitive to oxygen availability. In cortex, $\text{GABA}_{\alpha5}$ and $\text{GABA}_{\gamma3}$ subunits expression was reversed. Synaptic alpha5-GABA(A)Rs play a role in the phasic GABAergic inhibition of pyramidal neurons in hippocampus and cerebral cortex (Serwanski et al., 2006). $\text{GABA}_{\alpha1}$ and $\text{GABA}_{\delta}$ receptor subunits expression was decreased in the cortical region which may contribute to inhibition of cortical plasticity and associated learning impairments. An increased expression of $\text{GABA}_{\alpha1}$ mRNA was reported at the cortical site where the plastic changes were found which contributes to learning associated activation of the cerebral cortex (Lech et al., 2001). In brain stem, the seat of respiratory network, $\text{GABA}_{\alpha1}$ and $\text{GABA}_{\gamma3}$ receptor subunits expression was reversed to near control whereas $\text{GABA}_{\alpha5}$ and $\text{GABA}_{\delta}$ receptor subunits expression were decreased. Wei et al., (2003) reported a perisynaptic localization of $\delta$ subunit-containing GABA$_{\lambda}$ receptors in the brain stem and that these receptors are activated by GABA overspill in the molecular layer. $\alpha5$ subunit-containing GABA$_{\lambda}$ receptors mediate tonic inhibition and are important regulators of the expression of locomotor exploration (Hauser et al., 2005).

In epinephrine resuscitated groups $\text{GABA}_{\alpha1}$, $\text{GABA}_{\alpha5}$, $\text{GABA}_{\gamma3}$ and $\text{GABA}_{\delta}$ receptor subunits expression was reduced in cortex, cerebellum and striatum whereas in brain stem a differential subunit expression was observed. In the brain stem of hypoxic neonatal rats resuscitated with epinephrine alone, the expression of $\text{GABA}_{\alpha5}$, $\text{GABA}_{\gamma3}$ and $\text{GABA}_{\delta}$ receptor subunits was increased. A reversal in $\text{GABA}_{\alpha1}$ receptor subunit expression on epinephrine administration indicates the attempt of the CNS respiratory circuit to adapt to the diminished oxygen availability by enhancing cardiac pumping and other peripheral changes mediated by epinephrine. The expression of $\text{GABA}_{\alpha1}$ subunits in brain stem supports a role for GABA in the brainstem circuit controlling esophageal peristalsis (Broussard et al., 1996). In hypoxic neonates resuscitated with epinephrine along with glucose and oxygen, $\text{GABA}_{\gamma3}$ receptor subunit showed
increased expression which promotes tonic inhibition. Tonic inhibition was also produced by gamma subunit-containing GABA<sub>A</sub> receptors (Semyanov et al., 2004). Thus the difference in regional and subunit expression of GABA<sub>A</sub> receptors observed in the present study points to the effectiveness of glucose resuscitation to neonatal hypoxic rats in modulating the various respiratory responses in a molecular level.

**CENTRAL SEROTONERGIC RECEPTOR ALTERATIONS**

Serotonin plays a pivotal role in the control of breathing. Brain and spinal cord regions involved in respiratory control receive 5HT input primarily from the medullary raphe nuclei including nucleus raphe magnus, pallidus and obscurus (Holtman, 1988; Li et al., 1993; Manaker & Tischler, 1993). Caudal raphe neurons are activated during hypoxia, and presumably release 5HT in the vicinity of respiratory premotor and motoneurons (Erickson & Millhorn, 1994; Teppema et al., 1997; Kinkead et al., 2001). Serotonin has an excitatory effect on upper airway and phrenic motoneurons (Berger et al., 1992; Lindsay & Feldman, 1993; Di Pasquale et al., 1997). Serotonergic facilitation of excitatory drive in respiratory motoneurons is largely mediated by 5HT<sub>2</sub> receptors (Kubin et al., 1992; Lindsay & Feldman, 1993). One model of 5HT dependent plasticity in respiratory motor control is long term facilitation (LTF). LTF is a long lasting increase in respiratory motor output following either electrical stimulation of chemoafferent neurons (Fregosi & Mitchell, 1994) or intermittent hypoxia (Mitchell et al., 2001). The specific 5HT receptor involved appears to be of the 5HT<sub>2</sub> family mainly 5HT<sub>2A</sub> receptors (Kinkead & Mitchell, 1999; Fuller et al., 2001a; Mitchell et al., 2001). The serotonin transporter (5-HTT) plays a key role in central serotonergic neurotransmission by controlling its intensity and duration through the reuptake of 5-HT that has been released from serotonergic terminals, somas and/or dendrites.
Discussion

5-HT CONTENT IN THE BRAIN REGIONS.

Brain serotonin content increases in brain stem and cerebellum during neonatal hypoxia. This increase is due to an increase in the rate of 5-HT synthesis (Crandall et al., 1981). Changes in serotonin neurotransmission have demonstrated to alter 5-HT and 5-HIAA concentrations (Kwok & Juorio, 1987; Sandirini et al., 1997). In cerebellum and brain stem, conversion of tryptophan by pyridoxal phosphate increased the 5-HT content. Serotonin is metabolised to 5- hydroxy indole acetic acid (5-HIAA) by the mitochondrial enzyme monoamine oxidase (MAO, primarily MAO-A). The increase in 5-HT content in cerebellum and brain stem is brought about by significant increase in 5-HT synthesis and decrease in its breakdown to 5-HIAA. Caudal raphe neurons are activated during hypoxia, and presumably release 5HT in the vicinity of respiratory premotor and motoneurons (Kinkead et al., 2001). Serotonin has an excitatory effect on upper airway and phrenic motoneurons (Di Pasquale et al., 1997). Thus the elevated levels of 5-HT observed in the respiratory centers of brain is a response of the body to stimulate respiratory output from the lungs.

Cerebral cortex

In cerebral cortical development, early manipulations of the serotonergic innervation lead to altered development and plasticity in sensory areas in a variety of species (Gu & Singer, 1995; Osterheld-Haas & Hornung, 1996; Janusonis et al., 2004). Several studies have revealed the presence of 5-HT2A receptors in cortical pyramidal neurons (Willins et al., 1997; Amargos-Bosch et al., 2004; Santana et al., 2004). 5-HT influence the descending excitatory input into limbic and motor structures, where the prefrontal cortex projects through the activation of pyramidal 5-HT2A receptors.

The present study on binding parameters of total 5-HT and 5-HT2A receptors in the cerebral cortex showed that the receptor number was significantly increased in hypoxic neonatal rats. Even though the receptor affinity of 5-HT was
decreased, serotonin subtype 5-HT$_{2A}$ showed a higher affinity in hypoxic group. This suggests that the increase in 5-HT receptors may be directed towards 5-HT$_{2A}$ receptors, which protect the respiratory network against hypoxic impairment of brain function. Earlier studies reported changes in synthesis and metabolism of 5-HT during short-term (i.e. 30 min to 2 h) hypoxia (Davis & Carlsson 1973; Olson et al., 1983). A number of studies have demonstrated that serotoninergic system participates in the compensatory responses to hypoxia, such as hypothermia and hyperventilation (Poncet et al., 1997; Richter et al., 1999; Steiner & Branco 2002; McGuire et al., 2004; Gargaglioni et al., 2005). Richter et al., (1999) reported an elevation of extracellular 5-HT levels in the ventral respiratory group in response to hypoxic challenge, suggesting that 5-HT is involved in the elaboration of the HVR. The gene expression of 5-HT receptor and 5-HTT also showed a significant up regulation in hypoxic group. The increased 5-HT receptors with decreased affinity observed under hypoxic condition results in pulmonary vasoconstriction and hypertension as an adaptive response to the stress. The ability of 5-HT to prolong survival in anoxia revealed a 5-HT-activated metabolic pathway that liberates an alternative energy source (Shartau et al., 2010).

The alterations in 5-HT and 5-HT$_{2A}$ receptor status and 5-HTT was brought back to near control by the supplementation of glucose alone and glucose with oxygen to the sufferers of hypoxic insult during early neonatal period. The availability of glucose as a universal substrate for aerobic and anaerobic metabolism is of critical importance in cellular homeostasis. The enhancement of glucose transport has been deemed to be essential for the adaptive response to hypoxia. Hyperglycemic effects of 5-HT are closely related to the release of adrenaline from the adrenal gland, mediated by 5-HT$_{2A}$ receptors (Yamada et al., 1995). But 5-HT$_{2A}$ was also reported to decrease brain glucose utilization (Hommer et al., 1997). So during glucose resuscitation, 5-HT and 5-HT$_{2A}$ receptor function decreased and brought back to near control to facilitate maximum brain glucose utilization. Hypoxia induced ATP depletion causes a reduction in blood
Discussion

glucose levels which is encountered by glucose administration and immediate oxygenation helps in overcoming the anaerobic condition. Supplementation of 100% oxygen alone to hypoxic rats did not show any reversal in the receptor status and its gene expression to control. The supplementation of 100% oxygen generates reactive oxygen species (ROS) which have been well documented as causative mediators of excitotoxicity (Choi., 1988; Coyle & Puttfarcken 1993; Li *et al.*, 1998). Enzymatic oxidation of 5-HT at physiological pH forms tryptamine-4,5-dione (Wrona & Dryhurst, 1991). Tryptamine-4,5-dione is also formed *in vivo*, possibly by an oxidative enzyme or potent oxidizing agent, such as oxygen free radicals. If tryptamine-4,5-dione is formed *via* a free-radical reaction in the central nervous system, it participate in the pathological process of neuronal damage induced by ischemia or traumatic brain injury (Ikeda *et al.*, 1989; Sakamoto *et al.*, 1991). Supplementation of 100% oxygen cause oxidation of 5-HT, which along with free radicals can contribute to neuronal toxicity in this group.

It is a usual practice of clinicians to resuscitate neonatal hypoxia with epinephrine. The adverse effects of epinephrine supplementation, alone or even in combination with glucose and oxygen, was observed by studying the changes in 5-HT receptor, expression of 5-HT receptor and its transporter. The 5-HT and 5-HT$_{2A}$ receptor was significantly increased compared to control in epinephrine treated groups. The gene expression of 5-HT$_{2A}$ receptor and 5-HTT also showed a significant up regulation.

Epinephrine induces a hypoxia-neovascularization cascade and plays a primary role in vascular proliferation within soft tissues (Karacaoglu *et al.*, 2007). It is reported that repetitive hypoxic stress induced by labour is a powerful stimulus for catecholamine release in fetus and is accompanied by typical alterations of fetal heart rate (Jensen *et al.*, 2009). Epinephrine mediated platelet aggregation is potentiated by 5-HT (Shah *et al.*, 1999). Activation of serotonergic system on epinephrine supplementation has a synergistic effect on platelet aggregation, which negatively affects oxygen transport. Hence the increased 5-HT
function on epinephrine supplementation to hypoxic neonates facilitates platelet aggression, which in turn reduces the cellular oxygen transport to overcome hypoxia.

**Cerebellum**

The cerebellar neurotransmitters significantly differ with respect to ischemia and hypoxia, this response being directly related to the duration and intensity of the injury. Lee *et al.*, (2001) reported that hypoxic insult resulted in considerable neurocytological deficits of the Purkinje cells and altered glial fibrillary acid protein immunoreactivity in the fetal cerebellum. Ischemic hypoxia was reported to provoke alterations in the production system of nitric oxide in the cerebellum (Xu *et al.*, 1995). Cerebellum facilitate the respiratory response to hypoxia and that the fastigial nucleus is an important region in the modulation of the hypoxic respiratory responses, presumably via its effects on inputs from peripheral chemoreceptors (Carmeliet *et al.*, 1998). Maeshima *et al.*, (1998) reported a dense concentration of 5-HT$_{2A}$ receptors in rat cerebellum. Eddahibi *et al.*, (2000) showed that hypoxia in rats up regulates 5-HT transporter mRNA and it is required directly in the development of hypoxic pulmonary condition. Enhanced long term facilitation after chronic intermittent hypoxia involves an up regulation of a non-5-HT$_{2}$ serotonin receptor subtype or subtypes (Ling *et al.*, 2001).

A significant increase in total 5-HT and 5-HT$_{2A}$ receptor number and gene expression in the cerebellar region was observed in the present study under hypoxic stress. The confocal imaging of 5-HT$_{2A}$ receptor immunohistochemical analysis in cerebellum also showed increased pixel intensity under hypoxic condition. Hypoxia evidently mobilizes 5-HT and stimulates its biosynthesis. The increased 5HT helps in facilitating pulmonary vasoconstriction thereby equipping the body to encounter the stress. This will result in pulmonary hypertension in hypoxic neonatal rats. Serotonin is also reported to be important for effective
stimulation of cAMP levels and initiation of plasticity mediated by adenylyl cyclase (Lin et al., 2010).

The up regulated gene expression of 5HT$_{2A}$ and the increased receptor status was brought back to near control by the supplementation of glucose alone and glucose with oxygen to the sufferers of hypoxic insult during early neonatal period. Unlike the adult, where glucose supplementation prior to or during hypoxia-ischemia accentuates tissue injury, glucose treatment of perinatal animals subjected to a similar insult substantially reduces the extent of tissue injury (Baron et al., 1987). On supplementation of 100% oxygen alone to hypoxic rats, there was an increase in the receptor status and its gene expression. During 100% oxygenation 5-HT function increases to facilitate more blood flow and oxygenation to the cells. But the supplementation of 100% oxygen generates reactive oxygen species (ROS) which have been well documented as causative mediators of excitotoxicity (Choi et al., 1988; Coyle & Puttfarcken, 1993; Li et al., 1998). Both free radicals and glutamate has been suggested to be involved in tandem in the neurotoxicity induced by hypoxia, whereas glutamate alone is involved in ischemic neurotoxicity (Omata et al., 2000). NMDA neurotoxicity and oxidative stress have also been well documented as mechanisms underlying hypoxic–ischemic brain injuries. The adverse effects of epinephrine supplementation, alone and even in combination with glucose and oxygen was reported by studying the changes in 5HT receptor, expression of 5HT receptor and transporter.

**Brain stem**

Serotonin containing cell bodies are localized in mesencephalic and rhombencephalic raphe nuclei in the brain stem. It has been proposed that 5-HT could be involved in neuronal development and plasticity. Serotonergic fibres have a pervasive innervation of hypoxic-ischemic affected areas in the neonatal brain and serotonin is pivotal in numerous neurobehaviours that match many
hypoxic-ischemia induced deficits. Neonatal hypoxic-ischemia injury caused significant disruption of the brainstem serotonergic system that can persist for up to six weeks after the insult. The different vulnerabilities of serotonergic populations in specific raphe nuclei suggest that certain raphe nuclei underpin neurological deficits in HI-affected neonates through to adulthood (Reinebrant et al., 2010). The brain stem serotonergic system is important for early brain growth and development, including the development of the central respiratory rhythm (Richter et al., 2003). 5-HT depletion is concomitant with changes in nitric oxide synthase activity without affecting nitric oxide synthase expression in the dorsal raphe nucleus (Tagliaferro et al., 2001). Hypoxia decreased serotonin turnover (5-HIAA/5-HT) in the brain stem (Henley et al., 1992). In the central respiratory network, 5-HT\textsubscript{1A}R efficiently reduces the excitability of respiratory neurons as indicated by the suppression of hypoxic activation (Richter et al., 1999) and decreased ventilatory responses to hypercapnia (Taylor et al., 2005); thus, 5-HT\textsubscript{1A}R has inhibitory actions. 5-HT\textsubscript{2A}R on the other hand, has an excitatory role and animal studies have shown that 5-HT\textsubscript{2A}R receptor stimulation excites the respiratory motor system at the level of the pre- Botzinger complex resulting in increased gasping (Tryba et al., 2006) and increased frequency of two types of respiratory bursts representative of fictive eupneic activity and fictive sigh activity (Pena & Ramirez, 2002).

The binding studies of total 5-HT and 5-HT\textsubscript{2A} receptors showed a significantly increase in receptor number with an up regulation of its gene expression under hypoxic stress in the brain stem. The altered parameters observed under the stress was effectively reversed back to near control by resuscitating with glucose alone and in combination with oxygen. No significant reversal was found in hypoxic rats resuscitated with oxygen alone and epinephrine. The increased expression of 5-HT\textsubscript{2A} receptor excites the respiratory centers of the brain stem resulting in increased gasping and increased respiratory bursts. Since continuous innervation of 5-HT causes pulmonary vasoconstriction
and reduction of GABA receptors an effective resuscitation should bring back the elevated 5-HT receptor function to near control.

**Corpus striatum**

Broderick and Gibson (1989) reported that *in vivo* hypoxia increases rat striatal extracellular dopamine and to a lesser extent, extracellular serotonin. Furthermore, after repeated, mild hypoxic episodes or moderate hypoxia, the increases in rat striatal extracellular dopamine and serotonin continue even during normoxia. These studies support a role for dopamine and serotonin in hypoxic-induced changes in brain function. The hypoxic-induced elevation of these two neurotransmitters during normoxia are important in the production of hypoxic/ischemic-induced cell damage. Saligaut *et al.*, (1986) reported an inhibition of 5-HIAA formation and a complex interaction between synthesis, release and uptake of 5-HT in the hypoxic striatum and hypothalamus.

The present study reported an elevated 5-HT and 5-HT₂A receptor number under hypoxic stress in the striatum. The gene expression of 5-HT₂A and 5-HTT also showed a significant up regulation in hypoxic neonatal rats. Glucose supplementation reversed the alterations in striatal serotonergic receptors to near control. The immediate energy source from glucose helps in reducing the free radical toxicity thereby preventing the neurotransmitter alterations. Resuscitation with oxygen alone and epinephrine showed no reversal in the altered parameters indicating that 100% oxygenation and administration of epinephrine have worsened the neurotransmitter imbalance caused by neonatal hypoxic stress. 5-HT₂A receptor-mediated signaling events are strengthened within the striatum under conditions of dopamine depletion to provide a more potent regulation of motor activity (Bishop *et al.*, 2004).
CENTRAL MUSCARINIC RECEPTOR ALTERATIONS

Acetylcholine (ACh) is involved in the central control of respiration (Haji et al., 2000). Cholinergic systems originating in the pons (Dutschmann & Herbert, 1999) also control respiration in the sleep–wake states by affecting respiratory neurons and motoneurons (Bellingham & Berger, 1996; Bellingham & Ireland, 2002). Cholinergic control also occur through direct action on rhythm-generating neurons in the pre-Bötzinger complex, because muscarinic or nicotinic agonists affect these neurons and increase respiratory frequency when applied in the medullary slice in vitro (Shao & Feldman, 2000, 2001). Pharmacological evidence suggests that the muscarinic M1 and M3 receptor subtypes play a predominant role in respiratory control, primarily based on studies in the neonatal rodent brain stem preparation in vitro (Burton et al., 1994) and in the adult cat (Nattie & Li, 1990). The majority of respiratory neurons are cholinoceptive and are either excited or depressed by ACh or ACh agonists applied by iontophoresis in vivo, the proportion of depressed neurons being higher in anesthetized animals than in decerebrated preparations (Morin-Surun et al., 1984). It was shown that ACh increase respiratory frequency through muscarinic M3 receptor activation of rhythm-generating neurons in the pre-Bötzinger complex in the medullary slice in vitro (Shao & Feldman, 2000).

Cerebral cortex

In the central nervous system (CNS), the basal forebrain cholinergic system has been shown to be important in learning and memory. Cholinergic stimulation in regions of the brain such as cortex and hippocampus appear to have a modulatory role in facilitating responsiveness of cortical neurons to other inputs (Bartus et al., 1982). The RT-PCR and HPLC studies revealed that the muscarinic M1 receptor was present in a relatively high density in the cerebral cortex (Jian et al., 1994; Oki et al., 2005). It is hypothesized that the cerebral cortex participates in the memory, attention, perceptual awareness, thought, language and
Discussion

consciousness which are necessary for the normal life style. The muscarinic M1, M3 and M5 receptors are located predominantly on postsynaptic nerve terminals and are thought to be responsible for the role of the muscarinic cholinergic system in cognition and long term potentiation in the hippocampus and cortex (Bartus, 2000). Immunoprecipitation and immunofluorescence studies indicate that muscarinic M1 and M3 receptors are expressed in cortex (Levey, 1993).

Binding studies using $[^3H]QNB$ and muscarinic general antagonist, atropine revealed a significant decrease in total muscarinic receptor number in hypoxic neonatal rats. The decreased muscarinic receptors in the cortical region can attribute to the behavioural deficits observed in the later stages. Acetylcholine receptors are targeted to compartments rich in mitochondria, particularly postsynaptic domains and presynaptic terminals, exposing these receptors to reactive oxygen species. During neonatal hypoxic shock these receptors become exposed to ROS which resulted in a decrease in its number and affinity. Muscarinic AChRs are predominant cholinergic receptors in the central nervous system, where they play a major role in learning and memory (Gibbs, 1999), ingestion behavior (Rowland et al., 2003), and other central functions. Muscarinic M1/M3 receptors are involved in the control of tidal volume whereas M2/M4 receptors are involved in the control of breathing frequency and sensitivity to stress (Boudinot et al., 2008). The gene expression studies of muscarinic M1, M2 and M3 was down regulated in hypoxic neonatal rats indicating the reduction in tidal volume and breathing frequency under hypoxic stress.

Central cholinergic neurons participate in the complex neural events responsible for the hyperglycemic response to neurocytoglucopenia and to stressful situations. Atropine injected into the third cerebral ventricle suppressed epinephrine secretion and dose-dependently inhibited hepatic venous hyperglycemia induced by neostigmine in intact rats (Iguchi et al., 1990). The resuscitation with glucose alone and with oxygen effectively reversed the binding parameters and gene expression patterns to near control. Resuscitation with
oxygen alone and epinephrine did not show reversal of altered muscarinic function. Since glucose supplementation along with oxygenation provided an immediate energy source the imbalance in muscarinic function can be reversed by timely supplementation of glucose. Stabilising the muscarinic receptor function by glucose resuscitation improved the ventilatory response of hypoxic neonatal rats and reduced the behavioural deficits in the later stages of life.

Cerebellum

Cerebellum is a region of the brain that plays an important role in the integration of sensory perception, memory consolidation, coordination and motor control. In order to coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spinocerebellar tract (Roberta & Peter, 2003). There is currently enough anatomical, physiological and theoretical evidence to support the hypothesis that cerebellum is the region of the brain for learning, basal ganglia for reinforcement learning and cerebral cortex for unsupervised learning (Doya, 1999). The cellular basis of motor learning has been mostly attributed to long term depression (LTD) at excitatory parallel fiber - purkinje cell synapses. LTD is induced when parallel fibers are activated in conjunction with a climbing fiber, the other excitatory input to Purkinje cells. Recently, by using whole-cell patch-clamp recording from Purkinje cells in cerebellar slices, a new form of synaptic plasticity was discovered.

Total muscarinic receptor number showed a significant decrease with a down regulated expression of muscarinic M1, M2 and M3 receptor subtypes in hypoxic neonatal rats. Cerebellum participates in the learning and coordination of anticipatory operations which are necessary for the effective and timely directing of cognitive and non-cognitive resources (Allen et al., 1997). The cholinergic dysfunction, impaired glucose transport and oxidative stress contributes to learning and memory deficits in diabetes (Peeyush et al., 2010). The dimished acetylcholine muscarinic receptor function under hypoxic stress in neonates
contribute to memory and learning deficits. The current study revealed the modulatory function of glucose alone and along with oxygen on total muscarinic receptors by normalising the altered receptor gene expression and binding parameters to near control. The cerebellum has generally been suggested to be involved in the control and integration of motor processes, as well as cognitive functions. In the current study, we observed the neuroprotective effect of glucose on muscarinic receptors and muscarinic M1, M2 and M3 receptor subtypes in cerebellum, which is responsible for the coordination of voluntary motor movement, balance and equilibrium and declarative memory. Epinephrine administration and oxygenation alone was observed to worsen the situation by further decreasing the receptor function, which lead to behavioural deficits.

**Brain stem**

The neurotransmitter acetylcholine, acting through muscarinic receptors is involved in many aspects of respiratory neuromodulation (Haji et al., 2000), notably central chemosensitivity in brain stem structures (Ballantyne & Scheid, 2000; Burton & Kazemi, 2000). Pharmacological evidence, based primarily on studies conducted in vitro in the neonatal rodent whole brain stem (Burton et al., 1994) or brain stem slice (Shao & Feldman, 2000) and in the adult cat (Nattie & Li, 1990), suggests that the most important role in respiratory control is played by muscarinic M1 and M3 receptor subtypes.

The total muscarinic receptors of the brainstem were found to be decreased with a down regulated expression of muscarinic M1, M2 and M3 receptor subtypes in hypoxic neonatal rats. Muscarinic alterations in brainstem during neonatal hypoxia result in memory problems, difficulty in concentrating and difficulty in staying focused. Our results showed that glucose resuscitation restored the altered muscarinic functions associated with brainstem whereas resuscitation with epinephrine and oxygenation alone worsened the receptor functions.
Corpus striatum

Cholinergic terminals within the striatum contain presynaptic muscarinic receptors that inhibit neurotransmitter release (Chesselet, 1984). Various anatomical, electrophysiological and pathological observations provide evidence that ACh plays a major role in the control of striatal function and in the regulation of motor control (Jabbari et al., 1989). Striatal ACh is released from a population of large cholinergic interneurons that establish complex synaptic contacts with dopamine terminals, originating from the substantia nigra and with several striatal neuronal populations (Lehmann & Langer, 1982, 1983; Wainer et al., 1984; Phelps et al., 1985; Izzo & Bolam, 1988; Vuillet et al., 1992). Corpus striatum regulates endocrine functions indirectly through the secretion of other hormones like thyroxine.

Binding studies of total muscarinic receptors showed that the receptor number was significantly decreased with a down regulated expression of muscarinic M1, M2 and M3 receptor subtypes in hypoxic neonatal rats. The resuscitation with glucose alone and with oxygen effectively reversed the changed parameters to near control. Resuscitation with oxygen alone and epinephrine did not show reversal of altered muscarinic function. Since striatal acetyl choline receptor function is regulated through nigrostriatal pathway the changes in dopaminergic, serotonergic and GABAergic receptors influence the muscarinic function. The disturbances in the pathway resulted in an altered muscarinic status which contributes to the impairment in memory and cognition. The neuroprotective role of glucose resuscitation was also observed.

CHOLINERGIC ENZYME ALTERATIONS IN BRAIN OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Choline acetyltransferase (ChAT) is the rate-limiting enzyme of generating acetylcholine (ACh), which is synthesized in cholinergic neuronal cell
bodies and is often used in the studies of tissue localization and functional activity. The reduction of ChAT is correlated with the severity of dementia and pathologic changes (Rodrigo et al., 2004). Acetylcholine is the primary neurotransmitter of the cholinergic system and its activity is regulated by acetylcholine esterase (AChE). The termination of nerve impulse transmission is accomplished through the degradation of acetylcholine into choline and acetyl CoA by AChE (Weihua et al., 2000).

Central cholinergic activity was studied in the brain regions of experimental neonatal rats using ChAT and AChE as marker. The gene expression of ChAT in cerebral cortex, cerebellum, brain stem and corpus striatum was significantly down regulated under neonatal hypoxic stress. This indicates the drastic reduction in the anabolic pathway of ACh under hypoxic stress. Chang et al (2004) observed a down-regulation of ChAT immunoexpression in hypoxic rats which indicates the poor neurotransmission within the injured neurons. The pronounced reduction of ChAT in hypoxic rats result from a drastic shift of intracellular metabolic pathways, which in turn could lead to more metabolic loading to the severely damaged neurons. Severe hypoxic challenges are associated with decreased forebrain and brainstem ChAT immunohistochemistry and supression of hippocampal ChAT activity (Flavin et al., 1993; Tanaka et al., 1995; Nyakas et al., 1996). Acetylcholine and its receptors – both muscarinic and nicotinic are down regulated due to reduced synthesis of the neurotransmitter which lead to brain dysfunction in neonatal hypoxia. ACh prevented the hypoxia-induced apoptosis of mouse ES cells by inhibiting the ROS-mediated p38 MAPK and JNK activation as well as the regulation of Bcl-2, c-IAPs, and caspase-3 (Kim et al., 2008). Gibson and Duffy (2006) showed that even mild hypoxic hypoxia impairs ACh synthesis, which in turn account for the early symptoms of brain dysfunction associated with hypoxia.

The resuscitation with glucose and glucose along with oxygen to neonatal rats exposed to hypoxia effectively reversed the down regulated ChAT expression
to near control. Glucose forms the primary precursor for ACh synthesis. Supplementation of glucose provides the substrate for acetyl choline synthesis and also provides an immediate energy source to encounter ATP depletion. Resuscitating hypoxic neonatal rats with 100% oxygen showed a slight reversal in the expression of ChAT to near control. Epinephrine supplementation to hypoxic neonatal rats was not effective in restoring the altered ChAT expression. Epinephrine was reported to counteract completely the effect of acetylcholine in limited concentrations on contractile strength while only partially or weakly counteracting its effect on the action potential (Robert et al., 1960).

Acetylcholine esterase activity has been used as a marker for cholinergic activity (Ellman et al., 1961). AChE plays a very important role in the ACh-cycle, including the release of ACh (Kouniniotou- Krontiri & Tsakiris, 1989). The duration of action of ACh at the synaptic clefts is critically dependent on AChE activity (Cooper et al., 2003). In the present study, AChE expression in the brain regions was found to be up regulated in hypoxic neonatal rats. Extracellular and tissue cholinergic activity have been previously reported to be depressed during hypoxic insult (Freeman et al., 1987; Beley et al., 1991). Furthermore, hypoxia is reported to decrease the ACh content in the brain as a result of release of choline into extracellular space via activation of NMDA receptors (Zapata et al., 1998; Paulose et al., 2007) due to which the AChE expression was up regulated in neonatal hypoxic rats.

Glucose supplementation alone and along with oxygenation to hypoxic neonatal rats reversed the expression of AChE to near control. Decrease in the blood glucose level during hypoxia would lead to decrease in the brain ACh synthesis. Glucose, one of the main sources of acetyl CoA, is utilized by the cholinergic neurons to synthesize ACh (Willoughby et al., 1986) which reasonably explain the reversal of up regulated AChE expression in glucose supplemented hypoxic rats in our study.
Epinephrine supplementation and 100% oxygenation to hypoxic neonatal rats did not show reversal in AChE expression. This up regulation in the AChE expression in the brain regions of oxygen supplemented group is either due to the ROS induced tissue damage hindering the enzyme activity or due to decreased ACh content as a result of ROS. Epinephrine decreases the uptake of glutamate in the brain causing persistent activation of glutamate receptors (Paulose et al., 2007) which is capable of causing cholinergic dysfunction (Alkondon & Albuquerque, 2006) leading to a change in ACh content and AChE activity. Thus resuscitation with epinephrine is not effective in reversing the alterations in AChE expression in hypoxic neonatal rats.

SECOND MESSENGER ALTERATIONS IN CORPUS STRIATUM OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Cyclic AMP is generated through the action of adenyl cyclases, which is stimulated through appropriate G-protein coupled receptors (GPCRs) able to couple to the stimulatory guanine nucleotide regulatory protein, Gs (Wong & Scott, 2004). cGMP synthesis is catalyzed by guanylate cyclase (GC), which converts GTP to cGMP. Membrane-bound GC is activated by peptide hormones such as the atrial natriuretic factor, while soluble GC is typically activated by nitric oxide to stimulate cGMP synthesis. cGMP is a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis. In our studies we observed an elevated cAMP and cGMP level in the corpus striatum of hypoxic neonatal rats. The imbalance in the redox system of oxidative phosphorylation results in ROS production under hypoxic stress, which in turn activates the second messenger pathways as an adaptive modification. Millena et al (2006) reported that the increased cAMP levels in hypoxia are due to the ERK-mediated autocrine generation of prostaglandin E2. Consistent with such a role for ERK, MEK inhibitors was found to normalize cAMP levels in hypoxic hPASM cells presumably by curtailing this autocrine response (Millena et al., 2006).
Resuscitation with glucose brought back the altered cAMP and cGMP level to near control due to the reduced ROS production. Resuscitation with 100% oxygen forms ROS which triggers cAMP and cGMP production. In epinephrine resuscitated groups the cAMP and cGMP levels are high as epinephrine triggers cAMP formation. Epinephrine acts as a $\alpha_2$- and $\beta$-adrenoceptor agonist and $\alpha_2$-adrenoceptors interact with $\beta$-adrenoceptors and vasopressin receptors for cAMP accumulation (Yasuda et al., 1997). It was concluded that cAMP and cGMP play an important role in neonatal hypoxia, participate in the cellular signal transduction and promote the homeostatic response of the body to the stress.

IP3 functions by binding to the membrane-associated IP3 receptors (IP3R) (Berridge et al., 2003). Binding of IP3 to the receptor increases its sensitivity to Ca$^{2+}$, and only after Ca$^{2+}$ is bound can trafficking of the Ca$^{2+}$ into the cytosol take place. Notably, Ca$^{2+}$ has a biphasic action on the IP3R with a stimulatory effect at low Ca$^{2+}$ concentrations and an inhibitory effect at higher Ca$^{2+}$ concentrations (Nadif Kasri et al., 2002; Taylor & Laude, 2002). Acting as a signal transducer between two ubiquitous second messengers IP3 and Ca$^{2+}$, IP3R has been implicated in a variety of cellular and physiological processes as diverse as cell division, cell proliferation, apoptosis, fertilization, development, behaviour, memory and learning. In mammals, there are three distinct types of IP3R with splice variants observed among the types (Furuichi et al., 1994; Patel et al., 1999). IP3-receptor is dominantly expressed in neuronal cells throughout the central nervous system (Nakanishi et al., 1991; Furuichi et al., 1993). Throughout the brain, the IP3R1 is the predominantly expressed member of the family and its mRNA is widely distributed (Ross et al., 1992).

Our studies observed an elevated IP3 content in the striatum of hypoxic neonatal rats. The elevated IP3 level causes extra cellular release of Ca$^{2+}$, which in turn results in the activation of apoptotic pathways. Transfer of Ca$^{2+}$ between intracellular stores and mitochondria provides physiological control of respiration. But this Ca$^{2+}$ cycle also lead to cell death. If the matrix Ca$^{2+}$ level rises too high,
then deleterious changes in mitochondrial structure occur. In particular, mitochondria swell and rupture or undergo permeability transition, thereby releasing several pro-apoptotic factors into the cytoplasm, such as cytochrome C, second mitochondrial activator of caspases (SMAC/Diablo) or apoptosis-inducing factor (AIF) (Orrenius et al., 2003). This leads to the generation of the ‘apoptosome’ and activation of caspases from inactive zymogens. It is well established that Ca\textsuperscript{2+} released through IP3 receptors is sequestered by mitochondria (Rizzuto et al., 2004). Furthermore, it has been demonstrated that the flow of Ca\textsuperscript{2+} specifically from IP3 receptors can cause mitochondrial permeability transition and activate the apoptotic cascade (Szalai et al., 1999). Alterations in phosphoinositide-mediated signal transduction lead to the loss of mAChR sensitivity, which is also observed in the present study. Glucose resuscitation, alone and along with oxygen effectively brought back the elevated IP3 level to near control. The intracellular glucose level acts as a regulator of IP3 formation and signaling.

**EXPRESSION OF HYPOXIA INDUCIBLE FACTOR IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.**

HIF-1\(\alpha\) plays an essential role in cellular oxygen homeostasis by regulating the expression of genes involved in glycolysis, erythropoiesis and angiogenesis (Semenza, 2000\textsuperscript{a, b}). HIF-1 is a transcription factor specifically activated by hypoxia (Chavez & LaManna, 2002). The accumulation of HIF-1\(\alpha\) in ischemic or hypoxic tissues promote adaptive mechanisms for cell survival (Bergeron et al., 1999) and was found to be an important mediator of hypoxia-induced tolerance to ischemia (Bergeron et al., 2000; Jones & Bergeron, 2001; Bernaudin et al., 2002). Although HIF-1\(\alpha\) is essential for adaptation to low oxygen levels, it has also been shown in vitro to mediate hypoxia-induced growth arrest and apoptosis (Goda et al., 2003). HIF-1\(\alpha\) is also a key component of the cellular response to hypoxia and ischemia under pathophysiological conditions. HIF-1 is
reported to involve in the induction of cardioprotective molecules, such as inducible nitric oxide synthase (iNOS), hemeoxygenase 1 (HO-1), and erythropoietin (EPO), which in turn alleviate myocardial damages caused by harmful events such as ischemia-reperfusion injury (Tekin et al., 2010).

Our studies showed an up regulated HIF 1 expression in the cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats. The increased Hif 1 mRNA expression under hypoxia is suggested to be an adaptive response of the body to encounter the stress by facilitating angiogenesis, vasodialation and erythropoiesis. But in severe hypoxic cases, HIF-1α is accumulated and leads to cell death by activating different target genes (Semenza et al., 2000). It bind to pro apoptotic members of the Bcl-2 family such as BNIP3 (Bruick, 2000), Nix (Sowter et al., 2001) and p53 aswell as caspases (Li et al., 2005), which contribute to cell death or apoptosis. The role of HIF-1α in mediating pro death and pro survival responses, is dependent on the duration (Halterman & Federoff, 1999) and types of pathological stimuli (Aminova et al., 2005) as well as the cell type that it induces (Vangeison et al., 2008).

Suppression of HIF-1α is important for exploring HIF-1-dependent processes and for interfering with hypoxia-induced pathophysiological events. Hence the effectiveness of a resuscitation method for severe hypoxia depends on its capability to reverse the up regulated HIF expression. The present study reported a reversal in the elevated HIF gene expression in the brain regions by resuscitating the hypoxic neonates with glucose and oxygen. This points the significance of immediate resuscitation in encountering hypoxic stress without leading to cell death.

The traditional practice of epinephrine supplementation during prolonged hypoxia was found to up regulate the HIF expression to stimulate body functions that facilitate more oxygen transport to cells. Since HIF acts as a pro death response under severe hypoxia, epinephrine resuscitation can worsen the situation by activating cell death promoting pathways.
EXPRESSON OF BAX IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

The survival and effective functioning of the brain and brain cells are highly dependent on the balance between the brain’s ability to utilize oxygen and oxygen’s relative availability in the vascular system in the immediate surroundings. The human brain consumes some 20% of the body’s oxygen intake, but constitutes just 2% of total body weight (Erecinska & Silver, 2001). An extreme reduction of brain oxygen supply is known to lead to neuronal death (Hopkins & Haaland, 2004; Xu & LaManna, 2006), yet many situations of reduced oxygen do not lead to death of the organism, or even overt damage. Bax is a pro-apoptotic protein allowing apoptosis to occur through the intrinsic, damage-induced pathway and amplifying that one occurring via the extrinsic, receptor mediated pathway. Bax is present in viable cells and activated by pro-apoptotic stimuli. Bax has multiple functions: it releases different mitochondrial factors such as cytochrome c, SMAC/diablo; it regulates mitochondrial fission, the mitochondrial permeability transition pore; it promotes Ca^{2+} leakage through ER membrane (Ghibelli & Diederich, 2010). The expression of proapototic protein BAX can be taken as an index of cell death.

The present study observed a significant up regulation of Bax expression in the cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats. Bax is one of the key proteins that turn on the apoptotic cascade. The Bcl-2 family of proteins is an important determinant of apoptotic cell death. It consists of pro-apoptotic (Bax, Bcl-Xs, Bak and Bad) and anti-apoptotic (Bcl-2, Bcl-XL and Bcl-w) proteins (Adams & Cory, 1998). Bcl-2 family members determine cell death and survival by controlling mitochondrial membrane ion permeability, cytochrome c release and the subsequent activation of caspase (caspase 3, caspase 9) executor functions (Allen et al, 1998; Banasiak et al, 2000; Glasgow & Perez-Polo, 2000). Bax homodimers facilitate mitochondrial release
of cytochrome c via a process requiring Bax translocation to mitochondria (Crompton, 2000). The up regulated Bax expression in the present study indicates the high cell death in the cortical, cerebellar, straital and brain stem regions of neonatal rats exposed to hypoxic insult. The cell death in the brain regions in the early stage of development drastically affect the memory and cognition in the later stages of life. The standard approach to resuscitate neonatal hypoxia is to use 100% O$_2$. Further, resuscitation with 100% is recommended as a beneficial short-term therapy that is generally thought to be non-toxic (Martin et al., 2005; Kuisma et al., 2006). Although the use of 100% O$_2$ appears intuitive to maximize the gradient required to drive O$_2$ into hypoxic cells (Corff & McCann, 2005), a building body of evidence derived from animal models, has demonstrated that although resuscitation with 100% O$_2$ improves restoration of cerebral and cortical perfusion, occur at the price of greater biochemical oxidative stress (Martin et al., 2005). Further, results from investigations by Munkeby et al., (2004) suggest that resuscitation of asphyxiated piglets with 100% O$_2$ is detrimental to the brain. Our studies showed that the cell death in the brain regions of 100% O$_2$ resuscitated hypoxic rats is significantly high compared to control. This indicates that hyperoxygenation can trigger the apoptotic pathways resulting in cellular loss.

Immediate resuscitation with glucose, alone and along with oxygen, on the event of neonatal hypoxic stress was found to be effective in minimizing the cell death by down regulating the Bax mRNA expression. In the central nervous system, metabolic stress such as oxygen–glucose deprivation and hypoxic hypoglycemia in vitro deplete intracellular ATP stores and lead to the development of irreversible neuronal tissue damage (Lipton & Whittingham, 1979; Yoneda & Okada, 1989). Cell death caused by O$_2$ lack begins when anaerobic ATP production fails to meet the energetic maintenance demands of ionic and osmotic equilibrium. One of the earliest signs of neuronal responsiveness during a hypoxia-induced fall in ATP levels, coupled with extracellular accumulation of adenosine, a depression of synaptic transmission,
mainly due to presynaptic inhibition of L-glutamate release resulting from suppression of voltage-gated calcium currents (Krnjevic, 1999; Coelho et al., 2000). Hypoxia induced ATP depletion causes a reduction in blood glucose levels which can be encountered by glucose administration and immediate oxygenation helps in overcoming the anaerobic condition. Since glucose acts as an immediate energy source it can counteract the deleterious effects of ATP depletion induced cell death and other metabolic changes. In epinephrine supplemented groups the Bax expression was found to be up regulated in neonatal rats which points the adverse effects of epinephrine supplementation to hypoxic rats.

**EXPRESSION OF CREB IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.**

Cyclic AMP response element binding (CREB) protein, a transcription factor, mediates responses to a number of physiological and pathological signals such as neurotransmitters, synaptic activity, depolarization, mitogens, hypoxia and other stress factors (Sheng et al., 1991; Ghosh & Greenberg, 1995; Ginty, 1997; Vo & Goodman, 2001). CREB drives cell survival signalling (Walton et al., 1999; Walton & Dragunow, 2000; Ciani et al., 2002), and activation of muscarinic acetylcholine receptors promote cell survival. Indeed, many previous in vitro studies have shown that muscarinic agonists (primarily muscarinic M1 andM3, but also muscarinic M4) promote cell survival (Lindenboim et al., 1995; Yan et al., 1995; Itano et al., 1996; Budd et al., 2003; De Sarno et al., 2003; Budd et al., 2004; Wu & Wong, 2006). Interestingly, both the muscarinic receptor-mediated activation of CREB and cell survival appear independent of MAP kinase signalling (Leloup et al., 2000; Greenwood & Dragunow, 2002; Budd et al., 2003) suggesting that the CREB signaling pathway is involved in this cytoprotective effect. Previous studies of oligodendrocyte progenitors showed that stimulation of mAChR (Ragheb et al., 2001) phosphorylates CREB in a calcium-dependent manner (Pende et al., 1997; Sato-Bigbee et al., 1999). This transcription factor
promotes cell survival (Bonni et al., 1999; Watt et al., 2004) through the PI3K/Akt pathway to up regulate the expression of the anti-apoptotic factor Bcl-2 (Du & Montminy, 1998; Pugazhenthi et al., 2000).

In the present study the gene expression of CREB was down regulated in cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats compared to control. Even though cAMP level was increased in hypoxic neonatal rats, the CREB expression declined. Adaptive response of the body to hypoxia activates the second messengers to encounter the stress. But acute and prolonged hypoxia triggers the cell death pathways by activating pro apoptotic genes like bax, bad and destabilizing jun- fos complex. The activation of apoptotic pathways down regulates the CREB expression thereby blocking the cAMP signaling cascade in hypoxic neonatal rats. Down regulation of CREB is a consequence of apoptotic pathway activation and down regulation of muscarinic receptor function. These findings suggest that decreased CREB expression is the result of cell loss. The fact that CREB expression is known to be regulated in a number of systems (Brecht et al., 1994; Widnell et al., 1994; Walker et al., 1995) suggests that post-translational modification is not the only mechanism involved in the control of its trans-activation potential. Ca\textsuperscript{2+} increase induced by hyperosmotic stress promotes cell survival by recruiting CREB-mediated signaling. The fate of cardiomyocytes under hyperosmotic stress will depend on the balance between Ca\textsuperscript{2+} induced survival and death pathways by regulating CREB (Bordukalo-Niksic et al., 2010)

Resuscitation with glucose alone and along with oxygen reversed the down regulated CREB expression to near control. Hypoxic neonatal rats resuscitated with epinephrine or 100% oxygen did not show any reversal. Since glucose acts as immediate source of ATP, the hypoxic stress related ROS production and apoptosis is limited by administrating glucose to hypoxic neonatal rats.
EXPRESSION OF PHOSPHOLIPASE C IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL NEONATAL RATS.

Phospholipase C (PLC) mediates transduction of neurotransmitter signals across membranes via hydrolysis of phosphatidylinositol-4,5-bisphosphate, leading to generation of second messengers inositol-1,4,5-trisphosphate and diacylglycerol. In the CNS, neurotransmitter receptor coupling to phospholipase C has been extensively documented in [3H] inositol-labeled tissue slices and synaptosomes obtained from animal brains (Fisher & Agranoff, 1987; Stephens & Logan, 1989; Chandler & Crews, 1990).

In the present study, we observed hypoxia-mediated alterations in phospholipase C expression in the brain regions- cerebral cortex, cerebellum, brain stem and corpus striatum. Further we extended the studies to phospholipase C regulation with glucose, oxygen and epinephrine resuscitation for potential therapeutics which modulate signal transduction pathway for preventing CNS dysfunction in neonatal hypoxia. Our results showed an increased expression of phospholipase C in the cerebral cortex, cerebellum, brain stem and corpus striatum of hypoxic neonatal rats when compared to control. Muscarinic receptors M1, M3, M5 typically couple via α subunits of the Gq/11 family to activate phospholipase C (PLC), stimulating phosphoinositide (PI) hydrolysis (Caulfield & Birdsall, 1998). In particular, reconstitution experiments with purified muscarinic M1 receptors, G protein subunits and PLC suggested that the β1 subtype of PLC serves as the primary effector for the muscarinic M1 receptor (Felder, 1995). We considered that the up regulation of the Phospholipase C in the brain regions of hypoxic neonatal rats contribute to the increased IP3 levels in hypoxic rats. Phospholipase C performs a catalytic mechanism, generating inositol triphosphate (IP3) and diacylglycerol (DAG). Altered phospholipase C expression fails to modulate the activity of downstream proteins important for cellular signaling. Defective expression of phospholipase C causes the impaired release of Ca2+ and brings down the level of intracellular calcium and thus failed to execute the
normal neuronal function in the brain regions. During hypoxia fructose-1,6-bisphosphate initiates a series of neuroprotective signals which include PLC activation, small increases in \([\text{Ca}^{2+}]\) and increased activity of the MEK/ERK signaling pathway (Fahlmana et al., 2002). Previous studies report that phospholipase C-mediated signaling initiated by growth factor receptor types, are involved in long-term memory formation, a process that requires gene expression (Paul et al., 1999). These evidences led us to propose that the enhancement of hypoxia-mediated phospholipase C gene expression could impart damage to the central cognitive functions, which has been effectively protected by glucose resuscitation.

**BEHAVIOURAL DEFICITS IN HYPOXIC NEONATAL RATS**

The occurrence of hypoxic brain injury during fetal 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 (Saikumar et al., 1998; Delivoria-Papadopoulos & Mishra, 2000; Levison et al., 2001). Mild hypoxic–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). There has been much interest in the acute neurological changes associated with neonatal hypoxia, along with the mechanisms of subsequent central nervous system dysfunction in the adult (Lindahl et al., 1988; Berg, 1988; Soulier et al., 1997; Peterson, 2003). Hypoxia during the first week of life induce neuronal death in vulnerable brain regions usually associated with an impairment of cognitive function that can be detected later in life (Casolini et al., 2005).

We evaluated the spatial recognition and exploratory behaviour by Y-maze, spatial memory and learning by radial arm maze, memory and cognition by Morris water maze experiment in one month old rats exposed to neonatal hypoxia.
The reflex action and motor co-ordination was also evaluated by righting reflex test and wire maneuver test.

Y-maze is a simple recognition test for measuring spatial recognition memory. It is based on the innate tendency of rodents to explore novel environments (Dellu et al., 2000). In Y maze performance, the number of novel arm entries and time spent was significantly lower in hypoxic rats, showing the aversion for exploring new environments and defective spatial recognition. There are reports on the involvement of the cholinergic system abnormality in the impaired acquisition and/or retention of passive avoidance learning. In this respect, the observed behavioral abnormalities can be suggested to be a consequent of cholinergic dysfunction in hypoxic rats. However, in neonatal hypoxic rats resuscitated with glucose, alone and along with oxygen, the time spent and number of novel arm entries in the Y-maze was increased significantly. Y maze studies in epinephrine and 100% oxygen resuscitated groups showed decreased exploratory behaviour showing spatial recognition deficit. These findings indicate that glucose supplementation for neonatal hypoxia is an effective resuscitation programme in normalizing the cholinergic receptor dysfunction and thus improving the cognitive functions.

The radial arm maze is used primarily to measure spatial learning and memory. In radial arm maze, memory errors like working memory error and reference memory error were scored along with the number of trials needed to attain the criterion. Working memory is a transient form of memory that maintains task relevant information during conditions of competing demands (Baddeley, 1986). Working memory is operationalized as task accuracy in situations where information (or the appropriate response based on that information) changes frequently (i.e., from trial to trial), whereas reference memory reflects task accuracy when information (or the appropriate response based on that information) remains constant indefinitely (i.e. across trials and/or sessions) (Honig, 1978; Olton et al., 1979). The number of trials to attain five consecutive
criterion performances increased significantly in the hypoxic rats. Increased numbers of trials to criterion performance indicates the learning and memory deficit in hypoxic rats. A significant increase in memory errors was also scored in hypoxic rats indicating the impairment in coordination of tasks. High reference memory error in hypoxic rat points to the impairment with respect to procedural or reference memory i.e., what to do with the information they have. Glucose resuscitated rats showed a considerable reduction in the number of trials to attain the criterion performance and memory errors. The impairment in spatial learning, memory and task management was reversed by glucose supplementation to hypoxic neonatal rats, both alone and in combination with oxygen. This points the importance of a proper resuscitation programme to overcome neonatal hypoxia for a better intellect in the later stages of life.

The Morris water maze has been used extensively to measure cognitive deficits in brain-damaged rats (Brandeis et al., 1989). Performance in the Morris water maze depends on several elements, ranging from attention, learning and memory, to vision and motor coordination. Place navigation in the Morris water maze consists of two distinct components: declarative place representations as well as procedural learning (Morris et al., 1999). The procedural aspects include learning to inhibit inborn nonadaptive behaviour, such as swimming along the wall (Whishaw & Mittleman 1986; Paylor & Rudy 1990), while selecting appropriate behavioural strategies, such as swimming across the pool or uniformly searching its surface. Other procedural components involved skills such as improved distance and angle judgment that are a necessary prerequisite for the cognitive demands of the task. The hippocampal formation is critical for computing place representations but is believed to be dispensable for procedural memories (O’Keefe & Nadel, 1978). Morris water maze performance in one month old rats exposed to neonatal hypoxia showed the deficits in spatial memory and cognition in these rats. Hypoxic rats scored high escape latency with less time spent in the platform quadrant. A proper resuscitation to prevent neonatal death
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and adverse long-term neurodevelopment sequelae associated with neonatal hypoxia has to be established for better neonatal care. Radial arm maze, Y maze and Morris water maze tests showed that immediate resuscitation of neonatal hypoxia with glucose, alone and along with oxygen, showed an improved spatial recognition and memory in later stage of life.

The cognitive and memory deficits observed in one month old rats exposed to neonatal hypoxia is a consequence of a series of alterations in the brain regions at the molecular level. The decrease in GABA and muscarinic receptor subtypes function along with the activation of apoptotic pathways under hypoxic stress leads to functional disturbances in neonatal brain, which manifest in a later stage of life as behavioural deficits. GABA\textsubscript{A} receptors are involved in anxiety and convulsion (Matsumoto, 1989). where as GABA\textsubscript{B} receptors are related to depression and analgesia (Lloyd et al., 1985; Sawynok, 1990). GABA\textsubscript{A} as well as GABA\textsubscript{B} receptors play an important role in learning and memory (Castellano & McGaugh, 1991). GABA receptor systems modulate learning and memory through influences on cholinergic systems (Yutaka Nakagawa et al., 1995). Cholinergic system plays an important role during the early stages of memory formation (Naor & Dudai, 1996; Orsetti et al., 1996; Miranda & Berm_udez-Rattoni, 1999). Long-term potentiation (LTP) has been proposed as a model of induction of the synaptic plasticity underlying memory formation (Bliss & Collingridge, 1993; Escobar & Berm_udez-Rattoni, 2000). The neuronal plasticity needed for in vivo long-term potentiation is modulated by muscarinic acetylcholine receptors. Cholinergic system facilitate cortical plasticity by signaling stimulus relevance during memory formation. Improving the central cholinergic system and regulating the hippocampal monoamine neurotransmitters was reported to improve the learning and memory dysfunction (Wei et al., 2010). Since both GABAergic and cholinergic stimulation is important in learning, memory processing and cognition, along with the neuronal death the reduction in
GABAergic and muscarinic functions also contribute to the behavioural deficits observed in one month old rats exposed to neonatal hypoxia.

Evaluation of motor function by wire maneuver test showed that there is no significant difference in the grasping capability of hypoxic neonates with control and also within different groups. Righting reflex analysis showed that all groups of rats showed normal reflex action. In the present study, even though motor deficit was not observed due to hypoxic insult defective spatial memory and learning was observed by radial arm maze in 1-month old rats.

Hypoxic 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 (Mercuri et al., 2000; Shalak & Perlman, 2004). There has been much interest in the acute neurological changes associated with neonatal hypoxia, along with the mechanisms of subsequent central nervous system dysfunction in the adult. Hypoxia during the first week of life induce neuronal death in vulnerable brain regions usually associated with an impairment of cognitive function that is detected later in life. Postnatal hypoxia resulting from lung immaturity and respiratory disturbances in infants is an important pathophysiological mechanism underlying the devastating neurological complications. This points the importance of a proper resuscitation programme to overcome neonatal hypoxia for a better intellect in the later stages of life.

Thus our results showed that neonatal hypoxia contributes to CNS disturbances mediated through the functional regulation of GABAergic, serotonergic and muscarinic receptors. Also, gene expression of transcription factors- HIF1 and CREB, pro-apoptotic protein BAX, second messenger enzyme phospholipase C, anti-oxidant enzymes- superoxide dismutase and glutathione peroxidase and cholinergic enzymes were found to be altered in the CNS of hypoxic neonatal rats. Free radical scavenging capability, circulating insulin and triiodothyronine levels and second messengers- cAMP, cGMP and IP3 levels were
Discussion

also functionally altered by neonatal hypoxic insult. Resuscitation with glucose, alone and along with oxygen exhibited a potential effect in improving the ventilatory response and reversing the altered functional regulation of receptors, transcription factors, second messengers and enzymes of hypoxic neonatal rats to near control. The deleterious effect of oxygen alone and epinephrine resuscitation in neuronal response through alterations in neurotransmitters receptors functional regulation was also observed. Thus it is suggested that glucose administration immediately after hypoxia with oxygenated air as a resuscitation programme will be of tremendous advantage especially in neonatal care. Deeper understanding of the mechanisms through which neonatal hypoxia regulates the neurotransmitters, second messengers and transcription factors could point towards the development of new therapeutic approaches to reduce or suppress the pathological effects of hypoxia.