Literature Review

Hypoxia-ischemia (HI) occurring before or shortly after birth is a major cause of life-threatening injury and lifelong disability (du Plessis, 2002; Schubert et al., 2005). HI results in multi-organ failure and structural / functional damage especially devastating to the cardiovascular, renal, gastrointestinal and central nervous systems (Shah et al., 2004; Vento et al., 2005). The two most important causes of infant’s death were "intrapartum asphyxia and birth trauma" (intrapartum hypoxia) which resulted in neonatal "hypoxia" and "spontaneous preterm labour" leading to "immaturity related" births. Death rates in pre-term infants were particularly high in cities, towns and rural areas where neonatal care facilities are very limited. The large number of deaths associated with perinatal hypoxia suggested problems and inadequacies in care of women in labour and the resuscitation of newborn infants. There is probably poor insight into the deficiencies in the basic management of newborn infants as well as a lack of knowledge on neonatal resuscitation and care compared to intrapartum care (Robert et al., 2005).

Impact of Hypoxia

HI brain injury is very complex and has different neuropathological manifestations depending on the maturity of the newborn. As such, HI is etiologically linked to cerebral palsy, hearing and vision loss, mental retardation, learning disabilities, attention deficit hyperactivity disorder, schizophrenia and neuronal migration disorders (Johnston et al., 2002; Schubert et al., 2005). Brain is of special interest for hypoxia studies as it is extremely sensitive to reductions in oxygen supply. The brain damage occurs within a few minutes of hypoxia and result in severe and complex disabilities or death (Slavin, 1994). The reason for this vulnerability is that the brain has committed high energy costs that cannot be compromised. 50–60% of
the brain cells energy expenditure is devoted to transporting ions across the cell membranes in order to maintain cellular ion homeostasis (Lipton, 1999). As a result, the brain suffers energy failure even after few minutes’ interruption in oxygen supply. The anatomical consequences of neonatal hypoxia on the developing CNS vary from neuronal death (Dell’Anna et al., 1995) to altered neuronal differentiation. After acute hypoxia surviving immature neurons have a compromised neurite outgrowth and synapse formation. These minimal anatomical abnormalities underlie both behavioural-psychological dysfunction and neuroendocrine deficits (Nyakas et al., 1996).

Cerebral hypoxic ischemia appears to stimulate massive extracellular catecholamine release in the cortex, striatum and hippocampus. In vitro studies have also demonstrated elevated catecholamine concentrations and reduced uptake in gerbil synaptosomes during ischemia (Weinberger & Neives-Rosa, 1988). Central norepinephrine release during brain ischemia increases neuronal metabolism and exaggerates the discrepancy between impaired blood flow to ischemic tissue and an increase in the metabolic demand. Further, metabolism of excessive catecholamines leads to the formation of neurotoxic free radicals, whereas prevention of oxidative deamination of catecholamines reduces hydrogen peroxide production during reperfusion (Simonson et al., 1993). In addition to their direct detrimental effects, catecholamines also sensitize neurons to the excitatory amino acid glutamate, thus exacerbating the damage caused by glutamate during ischemia.

Thus, understanding the diagnosis, pathogenesis, resuscitation and treatment of those infants suffering hypoxic brain injury is paramount to reducing disability, improving survival and enhancing quality of life. Upon delivery, 5-10% of all newborns require some degree of resuscitation and assistance to begin breathing (Davis et al., 2004; Tan et al., 2006). The aim of resuscitation is to prevent neonatal death and adverse long-term neurodevelopment sequelae associated with a hypoxic event and rapidly reverse fetal hypoxemia and acidosis.
Hypoxia and neuronal death

Perinatal HI injury is accompanied by neurodegeneration, including features of both necrotic and apoptotic neuronal death as well as destruction of neurites connecting different neuronal populations (Ferrier, 2004). In studies with experimental animals, reducing neuronal death during and/or immediately after HI injury has been shown to markedly decrease long-term disability (Almli et al., 2000). Hypoxia is the key regulating factor that triggers inflammation as well as apoptosis in the human atherosclerotic plaque (Bitto et al., 2010). Hypoxia-reoxygenation injury is reported to induce apoptosis in neonatal rat cardiomyocytes (Zhang et al., 2010). Multiple cell death mediators have been reported to be activated in in vivo and in vitro models of neonatal HI injury, including various Bcl-2 family members, death receptors and caspases (Calvert & Zhang, 2005). The occurrence of hypoxic brain injury during fetal or neonatal development leads to damaged immature neurons and result in behavioral and/or cognitive dysfunction, including motor or learning disabilities, cerebral palsy, epilepsy or even death (Delivoria-Papadopoulos & Mishra, 2000; Levison et al., 2001; Saikumar et al., 1998).

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 is one of the key proteins that turn on the apoptotic cascade. The Bax protein was increased as a function of increase in degree of cerebral tissue hypoxia (Ravishankar et al., 2001). Hypoxia results in increased caspase-9 and caspase-3 activity in the cytosolic fraction of the cerebral cortex (Khurana et al., 2002; Mishra & Delivoria-Papadopoulos, 2006). Hypoxia results in increased fragmentation of nuclear DNA and the degree of DNA fragmentation increases as a function of
cerebral hypoxia (Akhter et al., 2001). Furthermore, it was shown that hypoxia results in generation of nitric oxide (NO) free radicals in the cerebral cortical tissue (Mishra et al., 2000) and administration of a nitric oxide synthase (NOS) inhibitor prevented the hypoxia-induced increased expression of Bax, caspase-9 activation, lipid peroxidation and increased fragmentation of nuclear DNA (Mishra & Delivoria-Papadopoulos, 2006; Numagami et al., 1997; Zubrow et al., 2002b). The neuronal death caused by neonatal hypoxic insult was mediated by the proteins involved in apoptotic pathways like Bax. Regulating the expression of these proteins can control the neuronal death due to hypoxic stress.

**Hypoxia and Medications**

Drugs are rarely indicated in resuscitation of the newly born infant (Burchfield, 1999). Bradycardia in the newly born infant is usually the result of inadequate lung inflation or profound hypoxia. Adequate ventilation is the most important step in correcting bradycardia. Administration of medications is required if, despite adequate ventilation with 100% oxygen and chest compressions, the heart rate remains <60 bpm. Epinephrine administration is indicated when the heart rate remains <60 bpm after a minimum of 30 seconds of adequate ventilation and chest compressions. Epinephrine is particularly indicated in the presence of asystole.

The standard approach of resuscitation for neonatal hypoxia is to use 100% O₂ (American Heart Association, American Academy of Pediatrics, 2005). Further, resuscitation with 100% is recommended as a beneficial short-term therapy that is generally thought to be non-toxic (Kuisma et al., 2006; Martin et al., 2005). Although the use of 100% O₂ appears intuitive to maximize the gradient required to drive O₂ into hypoxic cells, (Corff & McCann., 2005) a building body of evidence derived from animal models, has demonstrated that although resuscitation with 100% O₂ improves restoration of cerebral and cortical perfusion, it occurs 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\textsubscript{2} is detrimental to the brain. However in mice, resuscitation with 100% O\textsubscript{2} restores cerebral blood flow significantly faster than resuscitation with 21% O\textsubscript{2} and improves late neurofunctional outcome (Presti et al., 2006). Studies performed on asphyxiated human infants have shown that room air rather than 100% O\textsubscript{2}, favors clinical recovery (Vento et al., 2001\textsuperscript{a}; Vento et al., 2001\textsuperscript{b}).

Epinephrine has both $\alpha$- and $\beta$-adrenergic stimulating properties; however, in cardiac arrest, $\alpha$-adrenergic mediated vasoconstriction is the important mode of action (Zaritsky & Chernow., 1984). Vasoconstriction elevates the perfusion pressure during chest compression, enhancing delivery of oxygen to the heart and brain (Berkowitz et al., 1991). Epinephrine also enhances the contractile state of the heart, stimulates spontaneous contractions and increases heart rate. The recommended intravenous or endotracheal dose is 0.1 to 0.3 ml/Kg of a 1:10,000 solution (0.01 to 0.03 mg/Kg), repeated every 3 to 5 minutes as indicated. The data regarding effects of high dose epinephrine for resuscitation of newly born infants is inadequate to support routine use of higher doses of epinephrine. Higher doses have been associated with exaggerated hypertension but lower cardiac output in animals (Berg et al., 1996; Burchfield et al., 1993). The sequence of hypotension followed by hypertension likely increases the risk of intracranial hemorrhage, especially in preterm infants (Pasternak et al., 1983).

Epinephrine at a dose of 1 mg after every cycle of three unsuccessful shocks or after every three minutes of cardio pulmonary resuscitation during a non shock able arrest improves cerebral and coronary blood flow. In experimental animals, it increases peripheral resistance by adrenergic stimulation, thereby preventing arterial collapse during the release phase of cardiac compression. It also increases myocardial contractility and rate by $\beta$-adrenergic stimulation after restoration of an effective heart beat, or if in apparent PEA cardiac contraction is present but impalpable. Perhaps surprisingly, its benefit for survival in man is still debatable; no randomized controlled
trial has been attempted to support its use. High dose adrenaline has no clear advantage and is suggested to be deleterious (Vandycke & Martens, 2000).

**Neonatal Resuscitation during Hypoxia**

Neonatology, perinatology and neonatal resuscitation developed to a great extent during the 1970’s in response to an epidemic of litigation involving birth brain injury; foetal monitoring was detecting foetal distress *in utero* and specialized perinatal intensive care promised great improvement in neonatal morbidity and mortality. One third of all neonates receive some form of resuscitation treatment. About 6% to 10% of all neonates are “morbid” and need NICU care - many of these are premature. NICU mortality is extremely rare; however, in terms of neurological and mental disability, especially in NICU babies, long-term morbidity is anything but rare (Hack *et al*., 2002). The life saving procedures of neonatal resuscitation and NICU care are much less successful in preserving brains. Apgar score is a quantitative rating test with a maximum of ten used to measure the vital signs of a newborn a minute or so after birth: a score greater than seven signifies good health. Neurological impairment is likely if, resuscitation does not result in a five minute Apgar of 7 or more (Thorngren-Jerneck & Herbst, 2001).

The term “resuscitation” implies restoration of deficient life support systems, especially respiration; in the depressed newborn, that deficiency is in the placenta and cord, as the lungs have not yet begun to function. The rationale on which current resuscitation is based is that early detection of foetal asphyxia combined with rapid delivery and rapid establishment of pulmonary respiration (reversal of asphyxia) will prevent brain injury. If brain damage by neuron necrosis has occurred *in utero*, resuscitation will not heal it; however, overt brain damage seldom is evident at birth and it often appears after resuscitation. Hypoxic ischemic encephalopathy usually is diagnosed hours after birth when the child convulses; germinal matrix hemorrhage in
preemies (preterm babies) develop a day or two after birth; mental and behavioral problems will surface for years.

The general consensus is that birth “asphyxia” is the cause of the brain damage; hypoxia is a more precise term, although asphyxia implies arrest of respiration - respiration includes oxygen supply and removal of carbon dioxide. Iatrogenic resuscitation usually corrects this asphyxia promptly by initiating pulmonary ventilation; most organs survive superbly, except the brain. This strongly implies that there are other factors active in neonatal “depression” besides hypoxia and acidosis that must be corrected during “resuscitation”. The placenta is much more than a respiratory organ. Correction of the placental/cord deficiency that caused the depression and support of placental function are thus rational priorities in revival of a depressed neonate, just as they are in the “resuscitation” of the “distressed” foetus in utero.

In utero, the normal blood supply of the foetal brain is relatively hypoxic. Umbilical vein blood is fairly well oxygenated, but it is mixed in the inferior vena cava and in the heart with deoxygenated blood from the venae cavae; this is then circulated systemically. The color of a normal newborn is purple – it has been purple for nine months – circulating a mixture of haemoglobin (blue) and oxyhaemoglobin (red). It turns pink only after the foetal circulation is changed to the adult circulation and is combined with aeration of the lungs. The foetal brain thus grows and develops with a copious blood supply that is only partially oxygenated, but which readily removes products of aerobic and anaerobic respiration and excretes them through the placenta. The foetal kidneys and gut thrive on blood with the same oxygen partial pressure as the blood flowing to the placenta to be oxygenated. The newborn brain and other organs are therefore relatively immune to pure hypoxic injury (Kirks & Thorne, 1998) as long as organ and placental perfusion are copious.

The same basic principles apply to the adult brain; five minutes or more of cardiac arrest will produce some brain damage or brain death; occlusion of a cerebral
artery rapidly results in infarction (death) of the supplied tissue. On the other hand, five minutes or more of pure anoxia (e.g. breathing pure nitrogen) will produce unconsciousness that is fully reversible without brain damage provided that brain perfusion is not impaired. The integrity of the newborn brain is maintained (by perfusion and oxygenation) at normal (physiological) birth; therefore the physiological mechanisms that ensure these functions (perfusion and oxygenation) should be supported and/or duplicated during resuscitation if brain damage is to be avoided.

The severely depressed/asphyxiated newborn typically shows not only sign of breathing but also lack of muscle tone and reflexes needed to initiate breathing as well as signs of hypoxia such as cyanosis; in the most severe cases, pallor indicates vasomotor collapse. Such a child has obviously suffered a major respiratory insult prior to or during birth; the cause of that insult and its specific effects are factors that must be corrected, if possible, in the resuscitation process. In any and every case of newborn depression, if a child is born alive – with a heart beat and a pulsating cord – the placental life support system has not failed completely; utilization of this system in resuscitation and transition to “adult” life support systems in the depressed newborn is essential in restoring the physiological state – health – without the incursion of organ damage, primary or secondary, from “birth asphyxia.” With early detection of foetal distress and with rapid delivery, the neonate’s CNS should be undamaged at birth; the objective of therapy should be that it remains so.

The switch from placental to lung “breathing” is only a portion of the whole; the switch from placental alimentation and placental excretion to the newborn’s alimentary and excretory organs is also part of “natural” resuscitation. To initiate and establish the newborn functions of the lungs, gut, kidneys and other systems, including the brain, continuous copious perfusion of these organs is required; a large transfusion of placental blood during natural childbirth “resuscitates,” or more correctly “activates” all these organ systems as the massive flow of blood through the
placenta (40% of the foetal cardiac output) is diverted to these organs during physiological closure of the cord vessels.

Cord closure abruptly halts the placental supply of glucose to the brain (used in aerobic and anaerobic respiration); the neonatal liver (glycogen stores) must begin to maintain blood glucose levels. A major portion of the liver’s blood supply is from the hepatic portal vein that derives its blood from the mesenteric arteries. If the gut (and hence the liver) is not “copiously perfused,” hypoglycemia result in a neonatal convulsion. Deficient perfusion of the liver is also a factor in bilirubin excretion and “physiological” jaundice. Copious perfusion of the neonatal kidneys with adequate blood pressure is required for solute excretion, fluid, electrolyte and acid-base regulation after the placenta ceases to function. During the third stage of labor while the cord is pulsating, warm blood from the placenta courses through the newborn. After cord closure, temperature regulation is suddenly required of the neonate; switch of blood flow to and from the epidermis requires a copious amount of blood to regulate heat loss and heat retention.

In the foetus, pulmonary circulation is minimal; after the adult circulation is established, the entire cardiac output flows through the lungs. A major portion of the placental transfusion is utilized in establishing pulmonary blood flow after birth. Jaykka (1965) demonstrated that perfusion of the foetal lung “erected” the alveoli and actually initiated aeration; the high colloid osmotic pressure of the circulating blood rapidly absorbs amniotic fluid from the erected alveoli. Thus adequate “copious perfusion” of the lungs result in pulmonary oxygenation before any muscular respiratory effort occurs. Respiratory effort is reflexively controlled through the CNS; hypoxia and increased concentration of carbon dioxide are strong stimulants for receptors. For the reflex to function, copious perfusion of the reflex circuit is required, as is copious perfusion of the respiratory muscles (Jaykka, 1957).
**Free Radical Release and Toxicity**

Free radicals and reactive oxygen species (ROS) cause tissue damage only when the radicals exceed the brain’s endogenous antioxidant defences. Newborns and particularly pre-term infants are at high risk of oxidative stress and they are very susceptible to free radical oxidative damage. Free radicals are produced as a result of mitochondrial oxi-reductive processes and also produced by the action of enzymes such as xanthine/urate oxidase at extra-mitochondrial sites. These free radicals cause lipid peroxidations, especially in the cell membranes, inactivate cellular enzymes, inhibit nucleic acids and protein synthesis.

Free radicals or ROS are formed under hypoxic conditions. Antioxidants such as vitamin E can attenuate the effects of cerebral ischemia (Yamagata et al., 2010). Treatment of hypoxic pulmonary hypertension of rats includes stimulation of vasodilation of pulmonary artery and inhibition of oxidative stress (Fan et al., 2010). Cells have an enzymatic antioxidant pathway against ROS which are generated during oxidative metabolism: firstly, superoxide dismutase (SOD) catalyzes the formation of hydrogen peroxide from superoxide radicals, which is removed by a reaction catalyzed by catalase (CAT) and glutathione peroxidase (GPx) (Michel et al., 1994).

The neonatal brain is especially at risk of free radical mediated injury because neuronal membranes are rich in polyunsaturated fatty acids and the human newborn has a relative deficiency of brain superoxide dismutase and glutathione peroxidase (Buonocore et al., 2001). Normally, various antioxidant enzymes protect the body from these free radicals, but in hyperoxic situations, there is explosive free radical production leading to swamping of the enzyme systems and as a result free radicals escape inactivation (Chawla & Lavaniya, 2001). Roberto et al., (2005) reported that hyperoxia with 100% oxygen after hypoxia-ischemia cause more damage in the cerebral cortex than room air in newborn rats.

Flamm et al., (1978) correlated the generation of free radicals with cell damage in cerebral ischemia. Free radicals are highly reactive molecules that initiate
radical chain reactions and damage cellular macromolecules, including proteins, DNA and lipids, ultimately leading to cell death. Free radicals have been implicated in neuronal cell death in acute CNS injury and in chronic neurodegenerative diseases (Chan, 1994; Coyle & Puttfarcken, 1993). There are a number of potential sources for free radicals generation in the ischemic brain. This comprises leaks from mitochondrial respiratory chain; sequences catalyzed by cyclo-oxygenase and lipoxygenase, peroxidation of lipid membrane, auto-oxidation of various small molecules, including catecholamines, by the microsomal cytochrome P450 reductase system (Freeman & Crapo, 1982) and xanthine oxidase reactions. The brain and nervous system is especially prone to oxidative damage for a number of reasons (Ozben, 1998): the membrane lipids are especially rich in polyunsaturated fatty acid side-chains, which are prime targets for free radicals attack; the brain has only moderate amounts of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Also, it is relatively lacking in vitamin E; some areas of the brain are rich in iron ions which are released from injured cells or from bleeding in the reperfused area and enhancing lipid peroxidation. One particular role of oxygen free radicals in brain injury appears to involve reperfusion after cerebral ischemia (Chan, 1996). Reoxygenation during reperfusion provides oxygen to sustain neuronal viability and also provides oxygen as a substrate for numerous enzymatic oxidation reactions that produce reactive oxidants. In addition, reflow after occlusion often causes an increase in oxygen to levels that cannot be utilized by mitochondria under normal physiological flow conditions.

**Hormonal changes during Hypoxia.**

The endocrine system has an important role in the adaptation to hypoxia and stress and is reciprocally affected by these situations (Health & Williams, 1981). Hypoxia influences secretion of insulin and glucagon by regulating production of lactic acid which causes hyperinsulinemia and either up regulates or down regulates
secretion of glucagon depending on degree of hypoxia and glucose level. Adipocyte is highly expressed of insulin receptor, at the same magnitude as hepatocyte. Therefore growing plasma insulin by hypoxia is in favour for building-up of fat at adipocyte. Hypoxia imposes constant oxidative stress to islet beta cells which is by design in favour of oxidative phosphorylation. Insulin and muscle contraction stimulate glucose transport into muscle cells by separate signaling pathways, and hypoxia has been shown to operate via the contraction signaling pathway (Azevedo et al., 1995). Long term exposure to hypoxia plays a role in failure of beta cells which leads to diabetes mellitus (Linda & Nils, 2002). Hypoxia in adipose tissue contributes to obesity-related chronic inflammation, insulin resistance and metabolic dysfunction (Zhang et al., 2010). HIF-1alpha, the major transcription factor activated under hypoxia, is required for normal beta cell function and that dysregulation contribute to the pathogenesis of type 2 diabetes (Cheng et al., 2010)

Thyroid hormones are the most significant factors in regulating energy transformations in mammals by both short term (say few hours) and long term (say few days) effects (Hoek, 1992; Oppenheimer et al., 1985; Soboll, 1993). The calorigenic-thermogenic activity of thyroid hormone (T3) has been ascribed to uncoupling of mitochondrial oxidative phosphorylation (Yehuda-Shnaidman et al., 2010). In vivo, thyroid hormones are involved in setting the basal metabolic rate in many target tissues, such as liver, heart, kidney and brain. Many of the physiological actions of T3, the intracellular form of the hormone and its analogs are mediated through chromatin-associated nuclear thyroid hormone receptors (TRs), which are encoded by the a- and b- c-erbA protooncogene family (Apriletti et al., 1998; Lazar, 1993). TRa1 is a functional receptor that binds T3 with high affinity. TRa2 is a non hormone binding receptor that may act as a negative regulator. TRb1 and TRb2 differ in their N2- terminal sequences but both bind thyroid hormone and mediate its actions. TRa1 and TRb1 are expressed in virtually all tissues and are involved in regulating such diverse physiologic functions as metabolic rate, thermogenesis,
glucose utilization and organ development. TRβ2 is restricted to the brain and anterior pituitary gland where it plays a key role in mediating negative feedback by thyroid hormone on the hypothalamic-pituitary–thyroid axis. More than 100 gene products are required for assembly of the mitochondrial respiratory apparatus. These products are encoded primarily in the nucleus with only thirteen being coded on the mammalian mitochondrial genome (Attardi & Schatz, 1988).

Although T3 is considered to be a major regulator of mitochondrial respiration only nine of the nuclear-encoded genes have been shown to respond directly to thyroid hormone (Pillar & Seitz, 1997). This paradox has been resolved, at least in part, by the identification of nuclear-encoded transcription factors that are sensitive to stimulation by T3 and activate expression of respiratory genes (Weitzel et al., 2003). In addition, T3-binding sites directly on mitochondria have been identified consisting of truncated TRα1 isoforms (Bigler et al., 1992). The smaller version, p28, binds to the inner mitochondrial membrane. The larger version, p43, is localized to the mitochondrial matrix where it stimulates the mitochondrial transcriptional machinery in the presence of T3 (Casa et al., 1999). There have been no detailed studies on the effects of TH analogs on mitochondrial respiration or other non genomic actions, but they could have the potential to act through one or more of the mechanisms used by T3.

**Second Messenger changes during hypoxia**

Second messengers relay signals received at receptors on the cell surface to target molecules in the cytosol and/or nucleus. Three major classes of second messengers are (1) cyclic nucleotides (e.g., cAMP and cGMP), (2) inositol trisphosphate (IP$_3$) and diacylglycerol (DAG), (3) calcium ions (Ca$^{2+}$). The signal transduction in metabotropic neurotransmitters occur through activation of second messengers, whereas ionotropic neurotransmitters act through ligand gated ion
channels. The changes in neurotransmitter level and its receptor should agree with a concomitant change in second messenger for effective signal transduction.

**Inositol 1,4,5-trisphosphate**

Inositol 1,4,5-trisphosphate receptors are the IP3 gated intracellular Ca\(^{2+}\) channels that are mainly present in the endoplasmic reticulum (ER) membrane. Many biological stimuli, such as neurotransmitters and hormones, activate the hydrolysis of phosphatidyl inositol 4,5-bisphosphate, generating the second messenger IP3. The IP3 mediates Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores by binding to IP3 receptors (IP3R). The IP3 induced Ca\(^{2+}\) signaling plays a crucial role in the control of diverse physiological processes such as contraction, secretion, gene expression and synaptic plasticity (Berridge, 1993).

In mammalian cells, there are three IP3R subtypes, type 1 (IP3R1), type 2 (IP3R2), and type 3 (IP3R3), which are expressed to varying degrees in individual cell types (Taylor *et al*., 1999; Wojcikiewicz, 1995) and form homotetrameric or heterotetrameric channels (Monkawa *et al*., 1995). The confocal images of plasmid vector containing full-length rat IP3R3 linked to green fluorescent protein (GFP-IP3R3) provided strong evidence that IP3Rs are distributed preferentially on the ER network (Morita *et al*., 2002; Morita *et al*., 2004). Furthermore, Morita *et al*., (2004) demonstrated that the expressed GFP-IP3R3 acts as a functional IP3-induced Ca\(^{2+}\) channel. Frequently, IP3Rs are not uniformly distributed over the membrane but rather form discrete clusters (Bootman *et al*., 1997). The clustered distribution of IP3Rs has been predicted to be important in controlling elementary Ca\(^{2+}\) release events, such as Ca\(^{2+}\) puffs and blips, which act as triggers to induce the spatiotemporal patterns of global Ca\(^{2+}\) signals, such as waves and oscillations (Shuai & Jung, 2003; Swillens *et al*., 1999; Thomas *et al*., 1998). Tateishi *et al*., (2005) reported that GFP-IP3R1 expressed in COS-7 cells aggregates into clusters on the ER network after agonist stimulation. They concluded that IP3R clustering is induced by its IP3-
induced conformational change to the open state, not by Ca\textsuperscript{2+} release itself, because IP3R1 mutants that do not undergo an IP3 induced conformational change failed to form clusters. However, their results are inconsistent with studies by other groups (Chalmers et al., 2006; Wilson et al., 1998), which suggested that IP3R clustering is dependent on the continuous elevation of intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}). Thus, the precise mechanism underlying IP3R clustering remains controversial. Studies by Tojyo et al. (2008) have shown that IP3 binding to IP3R, not the increase in [Ca\textsuperscript{2+}], is absolutely critical for IP3R clustering.

Hypoxia results in a modification of the binding characteristics of the neuronal nuclear membrane inositol tetrabisphosphate (IP4) and inositol triphosphate (IP3) receptors. Mishra and Delivoria-Papadopoulos (2004) observed an IP4- as well as IP3-dependent increase in nuclear Ca\textsuperscript{2+} influx with increasing cerebral tissue hypoxia, suggesting a hypoxia-induced modification of the nuclear membrane IP4 and IP3 receptors.

**Cyclic Guanosine Monophospahte (cGMP)**

cGMP generation has been associated with neurotransmission (Hofmann et al., 2000), vascular smooth muscle relaxation (Fiscus et al., 1985) and inhibition of aldosterone release from adrenal glomerulosa cell suspension (Matsuoka et al., 1985). The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). One of the second messenger pathways, which have a role in learning and memory, is the NO–cGMP signaling cascade. NO–cGMP signaling is mechanistically involved in a number of animal models for learning and behavior, e.g. object recognition and passive avoidance (Baratti & Boccia, 1999; Bernabeu et al., 1996, 1997; Prickaerts et al., 1997; Prickaerts et al., 2002a, b; Rubin et al., 1997). cGMP effects are primarily mediated by the activation of cGMP-dependent protein kinases (PKGs). Two distinct mammalian PKGs, PKG-I and PKG-II, have been identified, as well as two splice variants of PKG-I (PKG-I\textalpha\ and -
In the brain, PKG-I is highly expressed in cerebellar Purkinje cells and, to a lesser extent, in striatal medium spiny neurons (De Camilli et al., 1984). PKG-II is a membrane-associated protein that is expressed throughout the brain (de Vente et al., 2001). The effects produced by the cGMP signaling pathway modulate drug-induced neural plasticity leading to behavioural alterations (Jouvert et al., 2004).

Activation of the NMDA receptor increases cAMP in the CA1 region of the hippocampus; this increase is mediated through Ca\(^{2+}\) calmodulin-dependent adenylyl cyclase (Chetkovich & Sweatt, 1993). The influx of Ca\(^{2+}\) also stimulates Ca\(^{2+}\) calmodulin-dependent nitric-oxide synthase (NOS) type to produce NO, which stimulates guanylyl cyclase to produce cGMP (Garthwaite, 1991).

Cyclic nucleotide pathways cross talk to modulate each other’s synthesis, degradation and actions. Increased cGMP increase the activity of cGMP stimulated PDE2 to enhance hydrolysis of cAMP, or it inhibit the PDE3 family and decrease the hydrolysis of cAMP (Pelligrino & Wang, 1998). cAMP and cGMP are involved in NMDA receptor-mediated signaling in cerebral cortical and hippocampal neuronal cultures. The influx of Ca\(^{2+}\) via the NMDA receptor stimulates calcium/calmodulin dependent adenylyl cyclase, leading to production of cAMP. This increase in cAMP seems to be tightly regulated by PDE4. The Ca\(^{2+}\) influx also stimulates the production of NO and subsequent activation of guanylyl cyclase, leading to cGMP production (Suvarna & O'Donnell, 2002).

**Cyclic Adenosine Monophosphate (cAMP)**

The second messenger concept of signalling was born with the discovery of cyclic AMP and its ability to influence metabolism, cell shape and gene transcription (Sutherland, 1972) via reversible protein phosphorylations. cAMP is produced from ATP in response to a variety of extracellular signals such as hormones, growth factors and neurotransmitters. Elevated levels of cAMP in the cell lead to activation of different cAMP targets. It was long thought that the only target of cAMP was the
cAMP-dependent protein kinase (cAPK), which has become a model of protein kinase structure and regulation (Canaves & Taylor, 2002; Doskeland et al., 1993; Francis & Corbin, 1999). In recent years it has become clear that not all effects of cAMP are mediated by a general activation of cAPK (Dremier et al., 1997). Several cAMP binding proteins have been described: cAPK (Walsh et al., 1968), the cAMP receptor of Dictyostelium discoideum, which participates in the regulation of development (Klein et al., 1998), cyclic nucleotide gated channels involved in transduction of olfactory and visual signals (Goulding et al., 1992; Kaupp et al., 1989) and the cAMP-activated guanine exchange factors Epac 1,2, which specifically activate the monomeric G protein Rap (Kawasaki, et al., 1998; Rooij et al., 1998).

**Transcription Factor changes during hypoxia**

Hypoxia selectively activates various transcription factors and proteins to overcome the ATP depletion induced brain damage and its consequences. Activation of cAMP responsive element binding protein (CREB) plays a key role in the lung-specific responses to hypoxia and that lung microvascular endothelial cells are important, proximal effector cells in the specific responses of the pulmonary circulation to hypoxia (Leonard et al., 2008). Hypoxia inducible factor (HIF) is another transcription factor important in the homeostatic regulation of hypoxia.

**Hypoxia inducible factor (HIF)**

The ability of cells to adapt to hypoxia is important for cell survival in both physiological and pathophysiological states (Bun & Poyton, 1996). Hypoxia inducible factor-1 (HIF-1) is a heterodimeric transcription factor which consists of HIF-1α, a subunit that is tightly regulated by cellular oxygen concentration and HIF-1, a constitutively expressed subunit (Wenger, 2002). HIF-1α plays an essential role in cellular oxygen homeostasis by regulating the expression of genes involved in glycolysis, erythropoiesis and angiogenesis (Semenza, 2000).
autonomous adaptive response to chronic hypoxia controlled by HIF-1 is reduced mitochondrial mass and/or metabolism. HIF-1 reduces ROS production under hypoxic conditions by multiple mechanisms (Semenza, 2010). Induction of HIF-1alpha expression increases the release of several cytokines, including proangiogenic mediators (Hatfield et al., 2010). Although HIF-1α 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-1α is also a key component of the cellular response to hypoxia and ischemia under pathophysiological conditions. Hypoxia-ischemia often results in severe brain injury in neonates (Ferriero, 2004). In neonatal brains, hypoxia-ischemia brain damage usually causes cell death through either necrosis or apoptosis. How the cell dies depends on the severity of the injury. Several studies have shown that apoptosis was more frequent in hypoxia-ischemia brain damage (Beilharz et al., 1995; Greijir & van der Wall, 2004). Apoptosis resulting from hypoxia is co-regulated by HIF-1α, as well as many other factors (Greijir & van der Wall, 2004). In adult mice lacking HIF-1α in brain cells, investigators found that the main role of HIF-1α in acute hypoxia is proapoptotic (Helton et al., 2005). On the other hand, in a rat neonatal stroke model with moderate ischemia–reperfusion, HIF-1α plays a neuroprotective role (Mu et al., 2003, 2005). Therefore, HIF-1α functions differently in regulating apoptosis in different animal models, species, or stimulus intensity.

**Cyclic AMP responsive element binding protein (CREB)**

The cAMP responsive element binding protein is a nuclear protein that modulates the transcription of genes with cAMP responsive elements in their promoters. Increases in the concentration of either Ca²⁺ or cAMP trigger the phosphorylation and activation of CREB. This transcription factor is a component of intracellular signaling events that regulate a wide range of biological functions, from spermatogenesis to circadian rhythms and memory. Evidence from *Aplysia,*
Literature Review

Drosophila, mice and rats show that CREB-dependent transcription is required for the cellular events underlying long-term but not short-term memory (Byrne, 1993). While the work in Aplysia and Drosophila only involved CREB function in very simple forms of conditioning, genetic and pharmacological studies in mice and rats demonstrate that CREB is required for a variety of complex forms of memory, including spatial and social learning, thus indicating that CREB is a universal modulator of processes required for memory formation (Silva, 1998). Beitner-Johnson and Millhorn (1998) reported that physiological reduction in $O_2$ levels induces a functional phosphorylation of CREB at Ser133 via a novel signaling pathway.

Neurotransmitter Receptors and their Role in Hypoxia

The brain neurotransmitter receptor activity and hormonal pathways control many physiological functions in the body. The pharmacological challenge strategy involves administering a test agent under controlled conditions to elucidate some aspect of biological or behavioural function in the organism being studied. It is based on the assumption that true functional abnormalities will not be evident in the basal state because of the action of compensatory mechanisms. Under such circumstances, pharmacological perturbation of a specific target system will reveal information about the functional integrity of both that system and systems that modulate it (Lawrence et al., 2000). Basing a treatment on symptoms alone (traditional medicine) will not provide the information needed to address the underlying brain imbalance. New sophisticated equipment and tests are now available to evaluate neurotransmitter imbalances using a urine or blood sample. This provides a neurotransmitter baseline assessment and is useful in determining the root causes for many diseases and illnesses. Laboratory analyses provide precise information on brain neurotransmitter deficiencies or overloads as well as detect hormonal and nutrient cofactor imbalances which influence neurotransmitter production. Testing helps to determine exactly which neurotransmitters are out of balance and helps to determine which therapies are
needed for an individualized treatment plan. It also helps in monitoring the effectiveness of an individual’s treatment.

**Adrenergic Receptors**

Adrenergic receptors belong to the large family of G-protein coupled receptors. These receptors form the interface between the sympathetic nervous system as well as many endocrine and parenchymal tissues (Hein & Kobilka, 1995). The adrenergic receptors contain seven stretches of 20-28 hydrophobic amino acids that represent membrane spanning regions. Adrenergic receptors are classified into α- and β-adrenergic receptors.

α₁-adrenergic receptors are activated by epinephrine (EPI) and norepinephrine (NE). α₂-adrenergic receptors mediate many physiological actions of the endogenous catecholamines, EPI and NE are targets of several therapeutic agents. EPI and NE are endogenous amines that are secreted in response to stress; they do not cross the blood-brain barrier. EPI is more potent agonist of β-adrenoceptors than α-adrenoceptors whereas NE is primarily an α-adrenoceptor agonist with some β-adrenergic activity. β-adrenergic receptor stimulation normally results in signaling by the heterotrimeric Gs protein, leading to the activation of adenylate cyclase, production of cAMP and activation of cAMP dependent protein kinase A (PKA).

If hypoxia is not too severe, coronary vasodilation enables myocardial O₂ delivery to balance increased myocardial O₂ demand in spite of reduced arterial O₂ content (Hermann & Feigl, 1992; Martinez *et al.*, 2005). The role of sympathetic activation in controlling coronary blood flow during hypoxia is complex, since sympathetic activation directly initiates both vasodilatory and vasoconstrictory mechanisms and indirectly initiates vasodilatory mechanisms. Central α -adrenergic receptor activity is important for fetal adaptation to hypoxia before birth. Endogenous inhibitory α-adrenergic receptor activation after severe hypoxia appears to significantly limit evolving hippocampal damage in the immature brain (Dean *et al.*, 2005).
Earlier studies reported an up regulation of \( \beta \)-adrenergic receptors during neonatal hypoxia while \( \alpha_2 \)-adrenergic receptors were down regulated. The up regulation was through the activation of cAMP pathway (Finla, 2007).

**Glutamate Receptors**

The majority of excitatory synapses are glutamergic, in which glutamate transmits the signal through postsynaptic ionotropic N-methyl-D-aspartic acid (NMDA), amino-3-hydroxy-5-methyloxazole-4-propionic acid (AMPA) and kainate (KA)] and metabotropic receptors (Bettler & Mulle, 1995). Glu is a fast excitatory transmitter in the CNS and has been shown, with GABA, to interact primarily with receptors in the synaptic cleft (Dingledine *et al*., 1999). The extracellular accumulation of glutamate results in neuronal death by activating ionotropic glutamate receptors sensitive to NMDA or AMPA–KA (Choi, 1988). The presence of G-protein coupled glutamate receptors (metabotropic Glu receptors) has been described. The metabotropic glutamate (mGlu) receptors are a family of eight G protein-coupled receptors that modulate cell excitability and synaptic transmission in the nervous system. Group I mGlu receptors stimulate release of \( \text{Ca}^{2+} \) from intracellular stores, which then modulates many signaling pathways, including those coupled to multiple receptor systems. Another type of interaction occurs at the second messenger level, where synergistic signaling is stimulated with simultaneous activation of receptors.

Glutamate functions as a fast excitatory transmitter in the mammalian brain. Recent experiments in a variety of preparations have shown that either blockade of synaptic transmission or the specific antagonism of postsynaptic glutamate receptors greatly diminishes the sensitivity of central neurons to hypoxia (Rothman & Olney, 1986). Glutamate triggers neuronal death when released in excessive concentrations by over excitation of its receptors (Vizi, 2000). Cell death due to excitotoxicity occurs in many types of cells in the newborn brain and the initial trigger will be impairment.
of the uptake of glutamate by glia, resulting in over activation of the receptors (McDonald & Johnston, 1990). It is reported that any sort of disturbances in the metabolic pathway of glutamate causes physiological and cognitive disorders (Preetha et al., 1996). Neurodegeneration by the glutamate receptor activation was reported to mediate motor dysfunction under hypoglycemia (Anu et al., 2010).

Hypoxia increases GABA levels in neurons by ATP depletion-induced activation of glutamate decarboxylase and by inhibiting GABA transaminase. Hypoglycemia, which also depletes ATP, reduces neuronal levels of GABA and its precursor Glu (Madl & Royer, 2000). Under hypoxia or ischemia, the release of aspartate, glutamate, glycine, alanine, taurine and GABA increased mainly by a Ca\(^{2+}\)-independent mechanism. However, ischemia highly potentiated the reduction of the energy charge, as compared with hypoglycemia or hypoxia alone. Addition of glucose metabolites, pyruvate and malate, attenuated neuronal death after exposure to glutamate or H\(_2\)O\(_2\) (Desagher et al., 1997; Ruiz et al., 1998).

**GABA Receptors**

Gamma-aminobutyric acid (GABA) was discovered over 40 years ago as a key inhibitory neurotransmitter in the brain (Bazemore et al., 1957; Krnjevic & Phillis, 1963). Since then, evidence has accumulated that this amino acid function as a neurotransmitter not only in the CNS but also in the peripheral nervous system, including the mesenteric plexus (Amenta, 1986), major pelvic ganglia (Akasu et al., 1999), sympathetic ganglia, encompassing the rat superior cervical ganglion (Kasa et al., 1988; Wolff et al., 1986) and abdominal prevertebral ganglia (Parkman & Stapelfeldt, 1993). In the mammalian central nervous system, GABA is the most important inhibitory neurotransmitter occurring in 30-40% of all synapses. Three types of GABA receptors have been identified: GABA\(_A\) and GABA\(_C\) receptors are ligand-gated Cl\(^-\) channels, while GABA\(_B\) receptors are G-protein coupled (Chebib & Johnston, 1999). GABA\(_A\) receptors are ligand gated Cl\(^-\) channels that consist of a
heteromeric mixture of protein subunits forming a pentameric structure and GABA<br>receptors couple to Ca2+ and K+ channels via G-proteins and second messengers<br>(Johnston, 1996). In the CNS, application of GABA reduces excitability by a<br>combination of GABA_A and GABA_B receptor activation, leading to membrane re-
polarization, reduced Ca2+ influx and suppression of neurotransmitter release. GABA_A<br>receptors are composed of five subunits from seven different subunit families with<br>multiple subtypes (α1–6, β1–3, γ1–3, δ, ε, θ, π) that form a ligand-gated chloride ion<br>channel.

The ventilatory response to hypoxia is influenced by the balance between<br>inhibitory (GABA, glycine and taurine) and excitatory (glutamate and aspartate) amino<br>acid neurotransmitters. GABA and glutamate are the two important neurotransmitters involved in Hypoxic Ventilatory Response. Decrease in ventilation during hypoxia in neonates is mediated through the effects of GABA on central nervous system. Decreased GABAergic function was reported under various stressful conditions like hypoglycemia and hyperglycemia (Antony et al., 2010) and CNS dysfunction like epilepsy (Mathew et al., 2010) which account for the increased vulnerability of brain to neuronal damage and motor learning deficits. 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). Tissue, perfused with artificial cerebrospinal fluid at 37°C with zero glucose and gassed with 95% nitrogen and 5% carbon dioxide, showed a five fold increase in glutamate release with little effect on GABA release. Pre-conditioning with three 5min periods of hypoxia/hypoglycemia preceding continuous hypoxia/hypoglycemia, significantly decreased glutamate release while significantly elevating GABA release. These results suggest that GABA reduce the release of glutamate and consequently decrease the neurotoxic effects of glutamate (Johns et al., 2000). Accumulation of GABA in hypoxic conditions results from sustained activity of glutamic acid decarboxylases (GAD), the enzymes that convert glutamate to GABA, but there is
concurrent inhibition of GABA breakdown by GABA transaminase A (GABA-T) (Hoop et al., 1999; Xia & Haddad, 1992).

**GABA<sub>A</sub> Receptor:**

GABA<sub>A</sub> receptors are pentameric in structure, with the five subunits arranged like spokes of a wheel around a central Cl<sup>-</sup> selective pore (Barnard, 2001). Nineteen GABA receptor subunits have been cloned from rats, which include α1–6, β1–3, γ1–3, ρ1–3, δ, 0, ε, and π (Whiting et al., 1999). The 19 subunits are encoded by 19 distinct genes. Each subunit has four transmembrane segments, with both the amino and carboxy termini located extracellularly. These extracellular segments form the recognition sites, two per channel, for GABA and also, in some channel types, the recognition site, one per channel, for benzodiazepine-like allosteric modulators. The genetic diversity of multiple GABA<sub>A</sub> receptor subunits permits the assembly of a vast number of receptor heteromeric isoforms. Apparently, the subunit composition determines the pharmacological profile of the resulting receptor subtypes (Barnard et al., 1998). Mechanisms that modulate the stability and function of postsynaptic GABA<sub>A</sub> receptor subtypes and that are implicated in functional plasticity of inhibitory transmission in the brain are of special interest (Luscher & Keller, 2004). Modification of GABA<sub>A</sub> receptor function has been implicated in a range of hypoxia-related pathologies, including encephalopathy (Low et al., 1985), seizures (Bergamasco et al., 1984) and myoclonus (Hallett, 2000).

GABA<sub>A</sub>R binding in gerbil hippocampus was reduced after hypoxia, which was thought to result from receptor internalization (Alicke & Schwartz-Bloom, 1995). It is reported that prolonged exposure to hypobaric hypoxia transiently reduces GABA<sub>A</sub> receptor number in mice cerebral cortex (Viapiano et al., 2001). GABA-mediated currents were reduced in CA1 pyramidal neurons in hippocampal slices exposed to hypoxia both in vivo and in vitro (Xu & Pulsinelli, 1994; Congar et al., 1995). Decreased GABA<sub>A</sub>R current in cultured hippocampal neurons subjected to
experimental ischemia was attributed to depletion of ATP and increased intracellular Ca^{2+} (Harata et al., 1997). Within 1 h after cerebral ischemia, GABA-gated Cl\(^{-}\) flux dramatically decreased in gerbil neurons (Verheul et al., 1993). Intracellular Cl\(^{-}\) in rat hippocampal slices was increased early after ischemia, resulting in reduced Cl\(^{-}\) driving force and GABA\(_{\Lambda}\) response (Inglefield & Schwartz-Bloom, 1998).

**GABA\(_{B}\) Receptor:**

The GABA\(_{B}\) receptor is part of the class C of GPCRs that also includes the mGlu, the Ca^{2+}-sensing and the sweet and umami taste receptors among others (Pin et al., 2003). These receptors are dimers, either homodimers linked by a disulphide bond (mGlu and Ca^{2+}-sensing receptors) or heterodimers made of two similar, but distinct subunits (the GABA\(_{B}\) and taste receptors). Indeed, the GABA\(_{B}\) receptor was the first G protein coupled receptor to be identified that requires two distinct subunits to function: the GABA\(_{B1}\) and GABA\(_{B2}\) subunits (Jones et al., 1998; Kaupmann et al., 2003; White et al., 1998). Although the GABA\(_{B1}\) subunit was soon shown to bind all known GABA\(_{B}\) ligands (both agonists and antagonists) this protein did not form a functional GABA\(_{B}\) receptor when expressed alone (Kaupmann et al., 1997).

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). Rhythm generation in mature respiratory networks is influenced strongly by synaptic inhibition. Zhang et al. (2002) reported that GABA\(_{B}\)-receptor-mediated postsynaptic modulation plays an important role in the respiratory network from post-natal day zero (P\(_{0}\)) on. GABA\(_{B}\)-receptor-mediated presynaptic modulation develops with a longer postnatal latency and becomes predominant within the first postnatal week (Suzuki et al., 1999). GABA\(_{B}\) receptors may contribute essentially to the modulation of respiratory rhythm in adult mammals and may be involved in the control of respiratory neuronal discharge (Ai-Lun Yang et al., 2007). In the elevated plus maze, the agonist of GABA-B receptor was reported to improve
consolidation of passive avoidance in rats undergoing hypoxia (Car et al., 2001). GABA$_B$ receptor-mediated activation of TASK-1 or a related channel provides a presynaptic autoregulatory feedback mechanism that modulates fast synaptic transmission in the rat carotid body (Ian et al., 2003).

**GABA$_C$ Receptors:**

GABA$_C$ receptors, which are a subfamily of GABA$_A$ receptors, are members of the Cys-loop superfamily of ligand-gated ion channels (LGICs), an important group of receptors involved in rapid synaptic transmission and whose malfunction results in a variety of neurological disorders; hence, understanding their mechanism of action is of considerable pharmacological interest. GABA$_C$ receptors are mostly located in retinal neurons where they play a role in retinal signaling involved in diseases such as macromolecular degeneration (Bormann, 2000). The receptors are activated by the binding of GABA, the main inhibitory neurotransmitter in the central nervous system. GABA$_C$ receptors have distinct pharmacological properties from GABA$_A$ receptors, e.g., they are not inhibited by bicuculline, the classic GABA$_A$ receptor antagonist (Barnard et al., 1998; Chebib et al., 2000). Like all the LGICs belonging to the Cys-loop superfamily, GABA$_C$ receptors are composed of five subunits arranged in a pentagonal array around a central ion-permeant pore. Each subunit has an extracellular N-terminal domain (ECD), a transmembrane domain composed of four $\alpha$-helices and an intracellular domain. Three subunits ($\rho_{1-3}$) have been identified; these all form functional homomeric or heteromeric receptors (Enz, 2001).

**Glutamic Acid Decarboxylase**

GABA the main inhibitory neurotransmitter in the brain is synthesized by glutamic acid decarboxylase (GAD). GAD exists in two isoforms termed GAD65 and GAD67 due to their molecular weights of 65 and 67 kDa, respectively. These enzymes are the products of two independently regulated genes sharing 65% sequence
homology in rats (Erlander et al., 1991a; 1991b). Most GABAergic interneurons express both subtypes of GAD (Esclapez et al., 1994; Houser & Esclapez 1994) which are simultaneously detectable in the rat brain as early as embryonic day 17 (Dupuy & Houser, 1996). GAD67 is found in axonal regions as well as in neuronal cell bodies, whereas GAD65 is mainly associated with synaptic terminals (Kauffman et al., 1991). Therefore it has been suggested that GAD67 mostly provides a pool of GABA for general metabolic activity while GABA synthesized by GAD65 is likely to be more involved in synaptic transmission (Martin & Rimvall, 1993). Mice lacking GAD65 are vital and do not exhibit changes in their brain GABA content though they have an increased susceptibility to seizures (Asada et al., 1996; Kash et al., 1997).

**Adenosine Receptors**

Adenosine is a powerful modulator of neuronal function, which mainly decreases the release of excitatory neurotransmitters and neuronal firing through the activation of inhibitory A1 receptors (Dunwiddie & Masino, 2001). Since adenosine is released in particularly high amounts in noxious situations, adenosine is conceived as an important endogenous neuroprotective agent against different noxious insults to the brain (de Mendonça et al., 2000). Adenosine, a purine nucleoside, is a neuromediator involved with many inhibitory mechanisms and thus regulates brain metabolism (Winn et al., 1981). Intracerebral concentrations of adenosine rise during hypoxia and are associated with increase in local cortical blood flow, decrease in whole-body oxygen consumption, reduction in Tb and protection against cerebral damage (Barros & Branco, 2000; Blood et al., 2003; Karimi et al., 1996; Koos et al., 1997; Rudolphi et al., 1992). Studies in anesthetized rats and in awake cats and lambs showed that adenosine is critically involved in the hypoxic ventilatory decline (Koos et al., 2004; Long & Anthonisen, 1994; Neylon & Marshall, 1991).
Blood et al., (2003) reported that in the near term foetal sheep, adenosine mediates a decrease of cerebral metabolic rate during acute moderate hypoxia via the adenosine A\(_1\) receptor activation. Furthermore, an inhibition of adenosine A\(_1\) receptors during severe asphyxia resulted in an increased neuronal cell death accompanied by delayed suppression of neural activity and increased cerebral metabolism (Hunter et al., 2003). Adenosine inhibits the evoked release of many neurotransmitters, both from peripheral nerves and in the CNS. The inhibitory effect of adenosine on NE (Fredholm & Dunwiddie, 1988) and ACh (Sperlágh et al., 1997) release has been particularly well described and proved to be mediated by adenosine A\(_1\) receptors. Adenosine A\(_1\) receptors have the general structure expected of G-protein-linked receptors and there is evidence that G\(_i\) proteins are involved in the inhibitory effects of adenosine on neurotransmitter release, inhibiting cAMP production and N-type Ca\(^{2+}\) channels and activating K\(^+\) permeability. In addition, there is some evidence that the activation of high-affinity adenosine A\(_{2A}\) receptors increases the release of different transmitters (Cunha et al., 1994; Gu & MacDermott, 1997; Sebastiao & Ribeiro, 1992) and has an effect on G\(_s\) protein and subsequently increases cAMP level. In contrast, its stimulation reduces the release of GABA from the recurrent collaterals of striatopallidal neurons (Kirk & Richardson, 1994).

**Serotonin Receptors**

5-HT receptors comprise a complex family. On the basis of their pharmacology, signal transduction mechanisms and molecular structure, more than a dozen types of 5-HT receptors have been identified (Hoyer et al., 1994). Most of these receptors are coupled to various G proteins with the exception of the 5-HT3 receptor, which is a ligand gated cation channel (Derkach et al., 1989; Maricq et al., 1991). Multiple 5-HT receptor subtypes are expressed in the cerebral cortex (Mengod et al., 1996). In cerebral cortex, 5-HT\(_3\) receptors are only expressed in inhibitory neurons (Morales & Bloom, 1997) whereas 5-HT\(_{2A}\) receptors are heavily expressed in
pyramidal cells and to a lesser extent in inhibitory neurons (Hamada et al., 1998; Jakab & Goldman-Rakic 1998; Willins et al., 1997). Since the 1960s, many experiments using in vivo microiontophoretic methods have characterized how 5-HT affects neuronal behaviour. The predominant effect of 5-HT on cerebral cortical pyramidal neurons is an inhibition of spontaneous spiking. (Jacobs & Azmitia, 1992; Phillis, 1984; Reader & Jasper, 1984). Intracellular studies in rat cortical slices suggested that 5-HT induces depolarization and action potential firing in pyramidal cells (Araneda & Andrade, 1991; Davies et al., 1987; Tanaka & North 1993). Furthermore, Aghajanian & Marek (1997) reported that 5-HT enhances spontaneous excitatory postsynaptic currents (sEPSCs) without significantly changing spontaneous inhibitory postsynaptic currents (sIPSCs) in frontal pyramidal neurons. These in vitro results suggest that 5-HT is mainly excitatory in cortical neuronal circuitry. 5-HT and α-methyl-5-HT had no effect on sEPSCs in layer I neurons. Even though sampling bias might have contributed to this observation, the fact that activation of 5-HT₂ₐ receptors induced robust enhancement of sEPSCs in all pyramidal neurons tested suggests that this differential modulation of sEPSCs in the two cell types was real. 5-HT₆ receptor expression is high in pyramidal neuron proximal apical dendrites and low in distal parts (Jakab & Goldman-Rakic, 1998; Willins et al., 1997). It is possible that activation of dendritic 5-HT₆ receptors induce dendritic transmitter release and/or release of retrograde messenger(s).

During brain development, serotonin provides essential neurotrophic signals (Justin et al., 2004). 5-HT is known to play an important role in several physiological functions (Jackson & Paulose, 2000). A root cause of sudden infant death syndrome (SIDS) is due to disturbances of serotonin levels in key pacemaker cells in the brain. In babies, the normal response to hypoxia is to gasp, which wakes the baby and resets the breathing mechanism. That reflex, which kicks in when a baby isn't getting enough oxygen for any reason, is governed by a set of pacemaker neurons in the respiratory neural network (Tryba et al., 2006). 5-HT is one of many vasoactive...
substances postulated to participate in the development of hypoxia-induced pulmonary hypertension. Pulmonary vasoactive responses to hypoxia are intensified by 5-HT (Eddahibi et al., 1997). Several subtypes of signal transducing 5-HT receptors have been characterized pharmacologically and cloned. Depending on their subtype, these receptors act on G-proteins and thereby activate phospholipase C or adenylate cyclase (Fanburg & Lee, 1997). By analogy with other signaling molecules, it is generally assumed that these receptors operate at the cell surface, without necessarily mediating the uptake of 5-HT. In addition, 5-HT is internalized into a variety of cell types, including platelets, neurons, mast cells, endothelial cells and smooth muscle cells, through an active transport mechanism that is powered by a transmembrane Na⁺/Cl⁻ gradient (Junod, 1972).

**5-HT₂₃ Receptors:**
Phrenic long-term facilitation (LTF) following acute intermittent hypoxia is a form of serotonin-dependent respiratory plasticity that requires 5-HT₂₃ receptor activation, new BDNF synthesis and activation of its high affinity receptor, TrkB (Baker-Herman et al., 2004). 5HT modulates the dynamics of the hypoxic sensory response via its action on 5-HT₂ receptors (Serrano et al., 2003). Abrea et al (2007) reported that 5-HT₂₃ receptors affect body temperature, ventilation and metabolism in hamsters. The role of 5HT₂₃ in glucose homeostasis and insulin secretion was reported from the studies in diabetic rats (Abraham et al., 2010a, b).

**Acetylcholine Receptors**
Acetylcholine is one of the principal neurotransmitters of the parasympathetic system. Extensive evidence supports the view that cholinergic mechanisms modulate learning and memory formation. Evidence for cholinergic regulation of multiple memory systems, noting that manipulations of cholinergic functions in many neural systems enhance or impair memory for tasks generally associated with those neural
systems. The magnitude of ACh release in different neural systems regulate the relative contributions of these systems to learning. ACh is the neurotransmitter that is released by stimulation of the vagus nerve, which alters heart muscle contractions. It is important for the movement of other muscles as well. ACh induces movement by the locomotion of an impulse across a nerve that causes it to release neurotransmitter molecules onto the surface of the neighbouring cell. ACh is critical for an adequately functioning memory. Studies of ACh release, obtained with *in vivo* microdialysis samples during training, together with direct injections of cholinergic drugs into different neural systems, provide evidence that release of ACh is important in engaging these systems during learning and the extent to which the systems are engaged is associated with individual differences in learning and memory (Paul, 2003).

Hypoxia impairs brain function by incompletely defined mechanisms. Mild hypoxia, which impairs memory and judgment, decreases ACh synthesis, but not the levels of ATP or the adenylate energy charge. The decreases in glucose incorporation into ACh and into the amino acids with hypoxic hypoxia (15% or 10% O₂) or hypoxic hypoxia with 5% CO₂ were very similar to those with the two lowest levels of anaemic hypoxia. Thus, any explanation of the brains’ sensitivity to a decrease in oxygen availability must include the alterations in the metabolism of the amino acid neurotransmitters as well as ACh (Gibson & Peterson, 1981).

**Muscarinic Receptors**

There are five subtypes of muscarinic ACh receptors (M1–M5) which belong to the superfamily of G-protein-coupled receptors. M1, M3, M5 receptors are mainly coupled to the Gq/11 protein which activates phospholipase C. Muscarinic M2 and M4 receptors are mainly coupled to the G/i/o protein, which inhibits adenylate cyclase.

There are at least three muscarinic receptor subtypes, M₁, M₂ and M₃ involved in the modulation of transmitter release (Caulfield, 1993; Caulfield & Birdsall, 1998).
This receptor diversity to some extent explain the diverse range of signal transduction mechanisms; these include inhibition of Ca$^{2+}$ influx (Allen & Brown, 1993, 1996), adenylyl cyclase, stimulation of guanylyl cyclase, activation of phospholipase C, direct inhibition of Ca$^{2+}$ channels and activation of K$^+$ channels (Felder, 1995). There is reasonably good evidence that the muscarinic M$_2$ receptors expressed on cholinergic (Allen & Brown, 1996; Aubert et al., 1995) and noradrenergic varicosities play a physiologically important role in the modulation of neurotransmitter release. The muscarinic receptors that inhibit NE release appear to be of the muscarinic M$_2$ subtype in the periphery and CNS. In contrast, there are muscarinic receptors, apparently of the muscarinic M$_1$ subtype, that increase the release of NE (Raiteri et al., 1990a, b) expressed on noradrenergic axon terminals in the periphery. The muscarinic M$_1$ receptor is generally coupled to PTX-insensitive G-protein. Its activation results in formation of inositol trisphosphate and diacylglycerol. In contrast, the muscarinic M$_2$ receptor is coupled via PTX-sensitive G-protein to the N-type Ca$^{2+}$ channel (Hille, 1992). The relative importance of these inhibitory and stimulatory muscarinic receptors vary in noradrenergic neurons from different locations. Cholinergic regulation of glucose utilization and cognitive functions was established in diabetic (Peeyush et al., 2010) and hypoglycemic (Anthony et al., 2010) conditions.

**Muscarinic M1 Receptors:**

Muscarinic M1 receptors are predominantly expressed in the forebrain, including the cerebral cortex, hippocampus and corpus striatum, where this sub-type contributes by 50-60% to the total of the muscarinic receptors (Gerber et al., 2001; Hamilton et al., 1997; Miyakawa et al., 2001). The muscarinic M1 receptor subtype, which is also expressed in peripheral tissues, has been implicated in stress adaptive cardiovascular reflexes and central blood pressure control. Studies have shown that central administration of the muscarinic M1 specific antagonist pirenzepine lowered the blood pressure (Brezenoff & Xiao, 1986; Buccafusco, 1996). A putative
overexpression of the muscarinic M1 subtype in selected brain areas of spontaneously hypertensive rats has been reported (Scheucher et al., 1991). Muscarinic agonist depolarisation of rat isolated superior cervical ganglion is mediated through muscarinic M1 receptors (Brown et al., 1980). Signaling through muscarinic M1 and M3 AChRs promote accumulation and transcriptional activation of HIF-1α. Muscarinic acetylcholine signals activate HIF-1 by both stabilization and synthesis of HIF-1α and by inducing the transcriptional activity of HIF-1α (Hirota et al., 2004). Perinatal hypoxia leads to an altered pulmonary circulation in adulthood with vascular dysfunction characterized by impaired endothelium-dependent relaxation and M1 AChR plays a predominant role. This implies that muscarinic receptors are key determinants in pulmonary vascular diseases in relation to perinatal imprinting (Peyter et al., 2008).

Muscarinic M2 Receptors:

Muscarinic M2 receptors mediate both negative and positive ionotropic responses in the left atrium of the reserpinized rat, latter effect being insensitive to pertusis toxin (Kenakin & Boselli, 1990). Central cholinergic transmission is activated by inhibition of the presynaptic M2 acetylcholine autoreceptor using selective antagonists. The presynaptic M2 autoreceptor negatively influences the release of acetylcholine in several brain regions, including the striatum, hippocampus and cerebral cortex (Billard et al., 1995; Kitaichi et al., 1999; Zhank et al., 2002). A direct consequence of brain muscarinic M2 autoreceptor inhibition is an elevation of acetylcholine release in the synaptic cleft. Methoctramine and other M2 receptor antagonists have been shown to enhance the release of acetylcholine in different brain structures (Stillman et al., 1993; Stillman et al., 1996). Muscarinic receptor activation in guinea pig heart produces a reduction in force of contraction and a decrease in the rate of beating. These effects are probably the consequence of inhibition of voltage-gated Ca²⁺ channels and activation of inwardly rectifying K⁺ channels, respectively.
Extensive studies with many antagonists have defined this response as being mediated by the muscarinic M2 receptors (Caulfield, 1993).

**Muscarinic M3 Receptors:**

Muscarinic M3 receptors are broadly expressed in the brain, although the expression level is not high, compared to those of the muscarinic M1 and M2 receptors (Levey, 1993). Muscarinic M3 receptor also triggers direct contractions of smooth muscle, however, it only represents a minor fraction of total muscarinic receptor population in smooth muscle. It is expressed in relatively low density throughout the brain. Studies using knock out mice for muscarinic M3 receptors gave evidences for the primary importance of these receptors in the peripheral cholinergic system. In urinary bladder, pupillary muscles and intestinal smooth muscles the cholinergic contractions are mediated predominately through muscarinic M3 receptors (Matsui *et al.*, 2000).

**Muscarinic M4 Receptors:**

Muscarinic M4 receptor is known to be abundantly expressed in the striatum (Levey, 1993). Muscarinic M4 receptors act as inhibitory muscarinic autoreceptors in the mouse (Zhang *et al.*, 2002). Inhibition of adenylyl cyclase activity by muscarinic agonists in rat corpus striatum is mediated by muscarinic M4 receptors (Caulfield, 1993; Olianas *et al.*, 1996).

**Muscarinic M5 Receptors:**

Muscarinic M5 receptor was the last muscarinic acetylcholine receptor cloned. Localisation studies have revealed that the M5R is abundantly expressed in dopamine-containing neurons of the substantia nigra par compacta, an area of the midbrain providing dopaminergic innervation to the striatum. Concordantly, oxotremorine-mediated dopamine release in the striatum was markedly decreased in
M5R-deficient mice. More intriguingly, in M5R-deficient mice, acetylcholine induced dilatation of cerebral arteries and arterioles was greatly attenuated (Yamada et al., 2001), suggesting that the muscarinic M5 receptor is suitable target for the treatment of cerebrovascular ischemia. Muscarinic M5 receptor subtype is expressed at low levels in the brain (Hosey, 1992; Hulme et al., 1990).

**Developmental Changes due to Hypoxia**

Hypoxia occurs when oxygen availability drops below the levels necessary to maintain normal rates of metabolism. Because of its high metabolic activity, the brain is highly sensitive to hypoxia. Severe or prolonged oxygen deprivation in the brain contributes to the damage associated with stroke and a variety of other neuronal disorders. Conversely, the extreme hypoxic environment found in the core of many brain tumours supports the growth of the tumour and the survival of tumour cells. Normal cells exposed to transient or moderate hypoxia are generally able to adapt to the hypoxic conditions largely through activation of the HIF. HIF-regulated genes encode proteins involved in energy metabolism, cell survival, erythropoiesis, angiogenesis and vasomotor regulation. In many instances of hypoxia and ischemia, the induction of HIF target genes is beneficial. When these same insults occur in tissues that are normally poorly vascularized, such as the retina and the core of solid tumours, induction of the same HIF target genes promote disease. Major new insights into the molecular mechanisms that regulate the oxygen-sensitivity of HIF and in the development of compounds with which to manipulate HIF activity are forcing serious consideration of HIF as a therapeutic target for diverse CNS disorders associated with hypoxia (Freeman & Barone, 2005).

From birth, exposure to a single hypoxic stimulus of 10/15 min duration induces an early peak in minute ventilation, followed by a reduction (roll-off), or hypoxic ventilatory depression. During early development, ventilatory responses to hypoxia tend to have a lower initial peak and more rapid decline compared to older
Role of Glucose in Regulating Energy Demand

Glucose is the major source of energy for organ function. In the human foetus, oxidation of glucose accounts for approximately 80% of foetal oxygen consumption, demonstrating that glucose is the major substrate for foetal oxidative metabolism (Jane & McGowan, 1999). Reports say that there occurs a close association between hypoxia and the emergence of glucose intolerance, but the experimental evidence of a causative role for hypoxia in this metabolic dysfunction is lacking (Oltmanns et al., 2004). Hypoxic respiratory diseases are frequently accompanied by glucose intolerance. One of the factors mediating this effect could be an elevated release of
epinephrine (Kerstin et al., 2004). The cerebral metabolic rate for glucose (CMRGlu) increased 70-80% after 2 min of hypoxia but then returned to nearly the normal rate by the end of the 30-min period of hypoxia. Glycolytic flux appeared to be facilitated in both groups initially but was inhibited as the hypoxic period continued. This slowing of glycolysis after 15 or 30 min of hypoxia appears to be modulated by the regulatory enzyme phosphofructokinase. A significant amount of the glucose entering the brain during the posthypoxic period appears to be used for metabolite synthesis rather than energy production (Kintner et al., 1983). Hypoxic-ischemic insult in the perinatal period in humans is a significant risk factor for the development of epilepsy later in life. Hypoxia is a leading cause of neonatal encephalopathy and is frequently associated with seizures (Jensen et al., 1991).

**Role of ATP in Regulating Energy Demand**

As a major consumer of energy, the brain is very susceptible to the effects of hypoxia, especially those parts of the brain – such as the hippocampus – that are crucial for cognitive function. There is no irreversible loss of neuronal/synaptic function, as long as nerve cells have an adequate supply of glucose and ATP (from anaerobic glycolysis) to maintain the minimal Na\(^+\)-K\(^+\) pump activity and protein synthesis essential for cell survival. These conditions are not met when both oxygen and glucose are deficient, as in strokes. Then the cell's protective mechanisms cannot cope with massive Ca\(^{2+}\) influx and it succumbs to the deleterious effects of Ca\(^{2+}\) overload (Krešimir, 1999). Of the approximately 130 million infants born worldwide each year, it is estimated that four million infants die during the first month of life. In animals, hypoxia is signalled at three levels: an immediate systemic response which involves central and peripheral chemoreceptors, an immediate/chronic gene response initiated by cellular oxygen signals and an immediate emergency or crisis response signalled by changes in energy metabolite concentrations (Peter & Howard, 2002).
ATP is a fast transmitter in sympathetic ganglia and at the sympatho-effector junction. In primary cultures of dissociated rat superior cervical ganglion neurons, ATP elicits noradrenaline release in an entirely Ca\(^{2+}\)-dependent manner. Nevertheless, ATP-evoked noradrenaline release was only partially reduced (by ~50%) when either Na\(^+\) or Ca\(^{2+}\) channels were blocked, which indicates that ATP receptors themselves mediated transmembrane Ca\(^{2+}\) entry (Stefan, 1999).

All kinds of biochemical reactions are linked to energy transfer, therefore each physiological function, as well as each pathological disorder or therapy, must have a consequence for biological energy. The adaptive changes related to hypoxia or energy deficit have been divided into defense and rescue phases. The defense phase occurs immediately after a decline in oxygen and consists of channel arrest, decreased Na\(^+\)/K\(^+\)-ATPase activity, urea synthesis, gluconeogenesis, protein synthesis and proteolysis (a highly ATP-consuming process), in such a way that ATP demand equals ATP production. Then the rescue phase involves transcriptional effects, HIF-mediated activation of genes for sustained survival at low ATP turnover (increased glycolytic enzymes, decreased enzymes involved in aerobic-linked metabolism) and finally production of tertiary cell signalling messengers - fos and jun. The consequences of cellular deficit and the mechanisms underlying adaptation to this situation can be understood from the results of numerous studies, both in hypoxia and in ischemia. Such adaptations must rely on a permanent adjustment between energy demand and ATP synthesis (Stefan, 1999).

**Effect of Hyper Oxygenation**

Oxygen availability plays a pivotal role in many cellular processes and therefore it is not surprising that most biological systems elaborate a variety of mechanisms for sensing oxygen and maintaining pO\(_2\) homeostasis (Lopez-Barneo et al., 2001; Semenza, 1999). In neuronal cells, responses to a decrease in oxygen availability or hypoxia include both facilitation and inhibition of neurotransmitter
release (Gibson & Peterson, 1981; Gibson et al., 1991). For example, hypoxia increase catecholamine releases (Hirsch & Gibson, 1984) or inhibits acetylcholine release (Freeman et al., 1987; Gibson & Peterson, 1981) from brain cells. In a peripheral chemosensory organ, the mammalian carotid body, hypoxia stimulates catecholamine release from specialized O$_2$-chemoreceptor (glomus) cells, whether present in the intact organ (Donnelly, 1993; Fidone et al., 1982), in tissue slices (Pardal et al., 2000) or as isolated cells or cell clusters in vitro (Jackson & Nurse, 1997; Montoro et al., 1996; Urena et al., 1994). Hypoxia also stimulates catecholamine release from neonatal adrenal chromaffin cells (Mojet et al., 1997; Thompson et al., 1997) and from PC-12 cells, an O$_2$-sensitive cell line derived from the adrenal medulla (Kumar et al., 1998; Taylor et al., 2000). In particular, hypoxia causes inhibition of K$^+$ channels, leading to increased membrane depolarization or action potential frequency, entry of extracellular calcium and amine secretion (Lopez-Barneo et al., 2001).

When blood supply and oxygen become compromised, local neurons die or become damaged in a pattern consistent with the injury. In this immediate area where blood and oxygen loss has occurred, the neurons die quickly. The surrounding neurons also react to the decreased oxygen levels by shutting down to conserve energy in an attempt to survive. This often results in an exaggeration of the symptoms experienced by brain-damaged patients. Presently, there is little information available on whether resuscitation using room air is equal to or even better than that using 100% oxygen (Nong et al., 2000). Newborns and particularly pre-term infants are at high risk of oxidative stress and they are easily susceptible to free radical oxidative damage. While no known treatments are yet able to resuscitate dead neurons, hyperbaric oxygen therapy (HBOT) serves to re-oxygenate the dormant neurons and restore a portion of their previous activity (Satokar et al., 1997). The clinical settings in which oxygen toxicity occurs are broadly divided into two groups; one is in which the patient is exposed to very high concentrations of oxygen for short duration, like in
HBOT and the second is in which lower concentrations of the oxygen are used but for longer duration. These two can result in the so called ‘acute’ and ‘chronic’ oxygen toxicity, respectively (Edmonds et al., 1992). The acute toxicity has predominant CNS effects, while chronic toxicity has predominant pulmonary effects (Clark, 1982). Hyperbaric medicine is considered extremely safe under appropriate supervision and utility. Toxic effects of oxygen are observed at extremely high doses over prolonged periods. Hyperbaric oxygen treatment increases the relative dose of oxygen; thus susceptible patients need to be recognized and modifications made to prevent the manifestations of oxygen toxicity. Oxygen derived free radicals had been suggested by Gerschman et al., (1954) as being the probable aetiological factor in the development of these toxic effects. Oxygen free radicals are reactive species that although crucial to normal biological processes can lead to injury and cell death. They are implicated in the pathogenesis of many neonatal diseases such as perinatal asphyxia, bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, intracranial haemorrhage, pulmonary hypertension and persistence of ductus arteriosus. Birth is associated with transition to a hyperoxic environment in comparison with uterine environment which leads to increased generation of free radicals. The newborn has undeveloped antioxidant systems and therefore at increased risk of free radical oxidative injury. The understanding of neonatal factors involved in the pathogenesis of "oxygen free radical diseases" will lead to the development of new therapies for prevention and treatment of these neonatal diseases (Rodrigues, 1998).

The key to successful neonatal resuscitation is establishment of adequate ventilation. Reversal of hypoxia, acidosis and bradycardia depends on adequate inflation of fluid-filled lungs with air or oxygen (de Burgh Daly, 1979, 1986). Although 100% oxygen has been used traditionally for rapid reversal of hypoxia, there is biochemical evidence and preliminary clinical evidence to argue for resuscitation with lower oxygen concentrations (Ramji et al., 1993; Rootwelt et al., 1993). Current clinical data, however, is insufficient to justify adopting this as routine practice. If
assisted ventilation is required, deliver 100% oxygen by positive-pressure ventilation. If supplemental oxygen is unavailable, initiate resuscitation of the newly born infant with positive-pressure ventilation and room air (Saugstad et al., 1998).

**Behavioural Changes Associated with Hypoxia**

Chronic hypoxia in advanced chronic obstructive pulmonary disease (COPD) result in altered and reduced neuropsychological functioning, which, in turn, leads to memory impairment even when other mental faculties remain unaffected (Sandhu, 1986). Multiple neuropsychological tests with these patients have revealed neuropsychological dysfunction, which is largely due to brain hypoxia. Reversal of cognitive dysfunction has been reported after oxygen therapy (Heaton et al., 1993; Krop et al., 1973) and even abnormalities in electroencephalograms have been shown to improve (Brezinova et al., 1979).

The temporal lobes and the hesh gyrus receive auditory information, modulate memory and language skills and relay information to the cortex where cognitive judgments are made and motor responses are integrated (Davidson & Irwin, 1999). The thalamus and basal ganglia act as relay stations between lower centres and the cortex (Kropotov & Etlinger, 1999). The brainstem enables endurance and survival capabilities, modulating heart rate, respiratory function and autonomic actions (Reid & Milsom, 1998). The pineal gland is thought to modulate sleep-wake cycles (Barrera-Mera & Barrera-Calva, 1998). The hippocampal area including the mammillary bodies modulates spatial memory formation, declarative memory, working memory, memory indexing/storage, relating expectancy to reality and internal inhibition. Memory is recorded in several parts of the brain at same time as 'memory molecules' for storage. These molecules are modulated by limbic system, especially the mammillary bodies. Bilateral hippocampal resection results in short term anterograde amnesia (Wise & Murray, 1999). The hippocampus has receptors for neurosteroids, both mineralocorticoid and glucocorticoid. The high affinity
mineralocorticoid receptors are agonized by aldosterone and antagonized by spironolactone. The low affinity glucocorticoid receptors are agonized by dexamethasone. There are no known antagonists to glucocorticoid receptors. The locus coeruleus is a small structure on the upper brainstem under the fourth ventricle and is involved in the regulation of wakefulness, attention and orientation (Smythies, 1997).

Some parts of the brain that are especially involved in higher cognitive functions (including consciousness) must be very dependent on a rich supply of energy – presumably because they are extremely active. In the first place, as their neurons continually generate many synaptic and action potentials, resulting in large inward and outward fluxes of ions, cellular and ionic homeostasis can be preserved only by the ATP-consuming Na\(^+\)-K\(^+\) pump, which maintains the trans-membrane Na\(^+\) and K\(^+\) gradients and thus indirectly supports such vital transport processes as uptake of sugars and amino acids. Even more than for the pump, 60% of ATP consumption is utilized for protein synthesis (Hochachka, 1996), presumably required to maintain the cell's structure, as well as the rapid turnover of enzymes, receptors and other proteins involved in neurotransmitter release, action and transport. These processes are crucial for synaptic transmission and plasticity and the closely related cognitive processes of memory, learning and selective attention. Hypoxia or hypoglycemia has almost immediate effects on behaviour and brain function. Though dramatic, they are fully reversible if the hypoxia or hypoglycemia is not sustained. But longer or more severe energy deprivation leads to irreversible functional and indeed cellular damage (cell death) – which develop only after a delay of some days (Pulsinelli et al., 1982). High incidence of minor neurological deficits, mainly regarding the fields of language and behaviour was reported in persistent pulmonary hypertension of the newborn (Berti et al., 2010). Extensive evidence indicates that peripheral or direct central glucose administration enhances cognitive processes in rodents and humans. These behavioural findings suggest that glucose acts directly on the brain to regulate neural
processing, a function that seems incompatible with the traditional view that brain glucose levels are high and invariant except under extreme conditions. However, recent data suggest that the glucose levels of the brain extracellular fluid are lower and more variable than previously supposed. In particular, the level of glucose in the extracellular fluid of a given brain area decreases substantially when a rat is performing a memory task for which the brain area is necessary. Together with results identifying downstream effects of such variance in glucose availability, the evidence leads to new thinking about glucose regulation of brain functions including memory (Ewan et al., 2002).

**Brain Wave Activity and Seizures as a Result of Hypoxia**

In severe encephalopathy there is an initial period of irritability or high arousal, often accompanied by seizures and apnoic spells, for the first 24 hours. The earlier the seizure, the more severe is the insult. This stage is followed by increasing coma with extreme hypotonia and progressive decline in brainstem function. Brainstem involvement is the best indicator of severe encephalopathy and the signs include abnormal eye movements and interference with sucking, swallowing which often persists as the bulbar and pseudo-bulbar palsy of the severe quadriplegic. Ongoing apnoea and cardio-respiratory arrest bring death at 2 to 3 days of age. When such cases “recover” the incidence of severe neuro-developmental abnormality is 100%. In a variety of clinical settings, an EEG-based monitoring system is considered to be optimal for the detection of an impending failure of cerebral oxygen supply (Prior & Brierley, 1980). In addition, there are other neurological structures implicated in cerebral palsy. The frontal lobe is in charge of voluntary motion. The left lobe controls the motor movements involved in language (speech and writing). The right lobe is usually involved in non-verbal activities. Damage to one frontal lobe usually results in a person's inability to move the opposite side of his body. Moreover,
damage to the frontal lobes can also cause the inability to initiate or respond to speech even though language can still be understood.

The parietal lobe is a structure where sensory information, such as touch, pressure, muscles, temperature and pain, is processed. Damage to one parietal lobe usually results in a loss of sensation in the opposite side of the body as well as being unable to feel touch, temperature and pain. The most frequent clinical syndrome, caused by lesions in the cerebral cortex and underlying white matter, is spastic paralysis (spastic cerebral palsy), which accounts for approximately 50% of all cerebral palsy cases (Miyahara & Mobs, 1995).

Seizures occur commonly in neonatal intensive care units (NICUs). They are an important clinical consequence of CNS diseases in the newborn including brain haemorrhage, stroke, meningitis and hypoxic-ischemic encephalopathy (Stephen et al., 2005). Seizures in the newborn are often clinically unsuspected. Consequently, the extent of the electrographic seizures burden in the sick baby can be greatly underestimated (McBride et al., 2000). A seizure affects the entire brain (generalized seizure), or it will be confined to one neural region (partial seizure). Autonomic changes are the most common symptoms of simple partial seizures but they go unrecognized. As effective seizure control in the neonate requires abolition of both clinical and electrographic seizures, EEG monitoring is necessary.

Neonatal seizures are paroxysmal alterations in neurological function. This can be behavioural, motor or autonomic (Volpe, 2000). Early pioneering work of 1970s by Wasterlain & Plum (1973) and Meldrum (1978) suggested that prolonged seizures and status epilepticus in mature and immature animals produced an energy failure leading to severe brain cell injury. Later experiments in the last 2 decades, however, seem to have disproved that theory by showing that 10 day old rats (equivalent to human newborns) maintain energy production in the brain by virtue of increased glycolysis and high adenosine triphosphate (ATP) release if there are no systemic complications such as hypoxia or hypotension (Ingvar & Siesjo, 1990). Most
of the literature about neonatal seizures concludes that the prognosis of a particular baby depends upon the etiology of the seizures. It is reported that certain etiologies, such as hypoxic-ischemic encephalopathy (HIE), meningitis, congenital brain abnormalities and inborn errors of metabolism, almost uniformly have severe neurological sequelae (Richard, 1999).

From infancy to adulthood, tonic–clonic seizures and complex partial seizures of temporal or extratemporal origin often lead to sympathetic activation. Because the memory circuits originate in the temporal lobe, repeated seizure activity which involves these structures cause difficulties with memory and intellectual function. Seizures typically activate sympathetic nerve activity, increasing the heart rate and blood pressure, although parasympathetic activation or sympathetic inhibition predominates during partial seizures (Orrin, 2004). Brain requires continuous supply of oxygen for energy utilization and efficient functioning. Hypoxia leads to disruption of this energy utilization, resulting in neuronal functional failure, cerebral palsy and neuro-developmental delay with characteristic biochemical and molecular alterations that can result in permanent or transitory neurological sequelae or even death. Structural and functional integrity of brain depends on regular oxygen and glucose supply.

In the present study, we investigated the role of glucose, epinephrine and oxygen resuscitation in GABA, serotonin, muscarinic receptors – their second messengers and transcription factors regulation in the brain regions of hypoxic neonatal rats. Gene expression studies using Real-Time PCR were done to analyse the changes in receptor, transporters, and transcription factor expression. Immunohistochemical studies using specific antibodies for the receptors were done in confocal microscopy to confirm the receptor data. The receptor pathway was studied by assaying second messenger levels and the proteins and enzymes involved in its pathway. The behavioural studies were done after one month in all experimental groups of neonatal rats to confirm the behavioural changes in later stages of life in
these rats. This study on hypoxic neonates shows that glucose supplementation has significant impact in controlling hypoxia induced functional damage to the neurotransmitter receptors and its signalling cascade, which has immense therapeutic application in the neonatal care.