Spinal cord injury is a devastating clinical problem that has permanent consequences. It has many medical, emotional and social consequences. It results in irreversible functional loss and lifetime disability (Sekhon & Fehlings, 2001). SCI is seen mostly in younger age group people (O'Connor & Murray, 2005). In the western countries, it is estimated that about 5 per 100,000 people suffer from the disabilities caused by SCI. The reasons of SCI are vehicular accidents (44.8%), fall from heights (21.7%), acts of violence like gun shots (16%) and sports injuries (13%). Since 80% of cases occur in younger age group between the ages of 16 to 30 years, SCI causes a significant cost in terms of lifetime care and loss of productivity.

Damage to motor nerves results in paralysis or loss of control of movement. Damage to somatosensory nerves results in loss of sensation and perception; one can no longer feel touch, pain, temperature or be able to tell without looking where in space the nerve damaged body part is positioned. After injury, the spinal cord undergoes a series of pathologic changes, including microhaemorrhage, cytotoxic edema, neuronal necrosis, axonal fragmentation, demyelination, secondary cellular destruction and eventually cyst formation (Balentine, 1978; Balentine & Greene, 1984; Coutts & Keirstead, 2008). The most frequent neurological deficit associated with SCI is incomplete tetraplegia (30.6%), followed by complete paraplegia (25.8%), complete tetraplegia (22.1%) and incomplete paraplegia (19.3%)

An injury to the spinal cord affects the brain regions (Gomez et al., 2012). When spinal cord is injured, there occurs a primary mechanical injury followed by a secondary injury. Primary injury is the initial mechanical damage, whereas secondary injury is progressive cell injury that begins in the gray matter and progresses into the white matter (Ballentine, 1978). Primary mechanical injury is
caused by the direct compression of spinal cord. Primary injury mechanisms include acute compression, impact, missile and distraction forces. The severity and the site of injury determine the effect of primary injury. The primary mechanical injury disrupts axons, blood vessels and cell membranes. Damage to blood vessels can be toxic to the CNS (Asano et al., 1980). It results in pathogenesis.

The concept of secondary injury was first put forward by Allen (1911). However, our knowledge of the exact mechanism through which primary injury triggers secondary injury is not very specific (Simon et al., 2009). The secondary injury phase involves vascular dysfunction, oedema, ischemia, excitotoxicity, electrolyte shifts, free radical production, inflammation and delayed apoptotic cell death. After SCI, the mammalian CNS fails to adequately regenerate due to intrinsic inhibitory factors expressed on central myelin and the extracellular matrix of the posttraumatic gliotic scar. Secondary injury disrupts the blood-spinal cord barrier and generates inflammatory response. Both barrier disruption and inflammation perturb the microenvironment and expose neurons to plasma-derived cells and molecules that can be injurious to intact, neighbouring tissue (Schlosshauer, 1993). Inflammation is considered to be an important element in secondary damage after SCI. This secondary damage leads to tissue loss and functional impairments. The immune responses are triggered by SCI and are mediated by a variety of factors that have both detrimental and beneficial effects. Inflammation that results from secondary injury is characterized by the accumulation of activated microglia, macrophages and contributes to secondary pathogenesis (Blight, 1992; Hirschberg et al., 1994; Popovich et al., 1994; Blight et al., 1995; Bethea et al., 1998). Inflammatory cells are coupled with delayed neuronal death and demyelination (Blight, 1985; Davis et al., 1990; Dijkstra et al., 1994; Hirschberg et al., 1994). Therefore, strategies have focused on diminishing the secondary effects of SCI (Dumont et al., 2001; Hall & Traystman, 2009;
Fehlings & Nguyen, 2010). SCI also leads to a range of alterations that are cytotoxic to both nerve cells and glial cells. Reports suggest that neurons and glial cells are changed permanently after SCI. Changes occur to segments above and below the SCI and these persistent changes lead to dysfunction. Hence, comprehension of secondary injury mechanisms and their complexities in SCI are invaluable requisite for planned therapeutic strategies: to stimulate axonal regrowth (regeneration), to arrest the self-perpetuating degeneration (neuroprotection), and the generation of new neurons and glia that will repopulate the site of injury and functionally integrate into the surviving neural tissue.

The most important physical consequences of a SCI are motor and sensory loss and impairments of bladder, bowel and sexual function leading to widespread disabilities in activities of daily life. There are certain health problems that arise secondary to the SCI. They are pain, spasms, pressure sores, urinary problems, bowel problems, respiratory failure, oedema and excessive sweating (Post et al., 1998). SCI is associated with respiratory complications. In case of acute SCI, 80% of cases are associated with respiratory complications (Tollefson & Fondenes, 2012). The common respiratory complications are atelectasis, pneumonia and respiratory failure (Jackson & Groomes, 1994). Pulmonary dysfunction is the cause for the largest portion of morbidity after SCI (Fishburn et al., 1990; Linn et al., 2000). SCI causes instant damage of nervous tissue followed by the loss of motor and sensory function. Due to the restricted self-repair ability of damaged nervous tissue, there underlies the need for reparative interventions to restore function after SCI. Without control from the brain, movements produced by a spinal CPG were not likely to be useful in restoring successful walking without regulation from the brain.

Magnetic Resonance Imaging (MRI) is the method to evaluate patients who have a persistent neurological deficit following SCI as it allows direct visualization of the injured cord, bony intervertebral and ligamentous structures,
and paraspinal soft tissues. MRI has replaced myelography and Computer Tomography myelography as the primary imaging preference available to assess compression of the spinal cord and is also a vital diagnostic modality in cases of SCI without radiographic abnormality. MRI also provides information regarding prognosis and neurological recovery.

CURRENT TREATMENTS AND ITS SIDE EFFECTS IN SPINAL CORD INJURY

There are various treatments available for SCI. Corticosteroids are used for the pharmacological treatment of SCI. They act by improving the blood flow in the spinal cord, restore impulse transmission, regulates calcium metabolism and enhance functional neurological recovery (Anderson et al., 1982; Bracken, 1992; Hall, 1993; Constantini, 1994). Methyl prednisolone is a corticosteroid that has anti-oxidant activity and is used in SCI treatment (Bracken, 1990). A study by Yu et al., (2004) reported that early repeated methyl prednisolone sodium succinate treatment allows greater recovery from SCI. Sharma et al., (2004) suggested that methyl prednisolone sodium succinate was of use in promoting post traumatic clinical recovery when given 1h after trauma. Methyl prednisolone sodium succinate proved to be more effective than dexamethasone in reducing edema when both are given after an interval of 1h (Sharma et al., 2004). Methylprednisolone also has a neuro protective effect (Amar & Levy, 1999). It improves neurologic function when given within 8 hours after injury (Bracken et al., 1990). It also has various side effects. It causes severe allergic reactions (rash, hives, itching, difficulty breathing, tightness in the chest, swelling of the mouth, face, lips, or tongue, unusual hoarseness) bloody black or tarry stools, changes in body fat, chest pain, fainting, fever, chills or sore throat, increased hunger, thirst or urination, mental or mood changes (eg, depression, personality or behavioural changes), muscle pain, weakness or wasting, seizures, severe nausea or vomiting, shortness of breath, slow fast or irregular heartbeat, slow wound healing, stomach
pain, sudden severe dizziness or headache, swelling of the feet or legs, tendon bone or joint pain, thinning or discoloration of the skin, unusual bruising or bleeding, unusual skin sensation, unusual weight gain, vision changes or other eye problems and vomit that looks like coffee grounds.

Lazeroids lack gluco corticoid activity and inhibits free radical formation. They also inhibit lipid peroxidation and arachidonic acid formation (Quarles et al., 1990). Endogenous opioids, acting through opiate receptors within the spinal cord, mediate certain secondary pathophysiological changes that contribute to irreversible tissue injury. Opiate receptor antagonists also reduces the secondary damage that occurs after SCI (Faden & Salzman, 1992). Hyperglycaemia increases reactive acidosis and triggers biochemical events such as increase in calcium levels and break down of cell membrane that lead to neuronal death (Sala et al., 1999). Calcium plays a key role in neuronal injury. Therefore calcium channel blockers are used to treat SCI. Nimodipine is one such calcium channel blocker that increases the rat spinal cord blood flow. Free radical scavengers and anti-oxidants are also used to treat SCI (Hall, 1992). GM1 Ganglioside, a complex acidic glycolipid compound present in the neuronal membrane is also used in the treatment of SCI (Geisler et al., 1991). Adenosine also has a neuro protective effect on SCI (Sulfianova et al., 2002). Levetiracetam prolonged the survival and the function of spinal motor neurons and have a therapeutic potential for several diseases that kill or degenerate the spinal motor neurons, including SCI (Yasuhiro et al., 2012).

Clinically existing treatments provide modest benefit; therefore present research is aimed at developing more effective therapies for spinal cord repair and regeneration (Kwon et al., 2004; Baptiste & Fehlings, 2007; Ali & Bahbahani, 2010; Fehlings & Nguyen, 2010). None of the human trials has produced a major progress in neurological recovery or a meaningful increase in function (Tator, 2006; Simon et al., 2009; Wang et al., 2009; Jablonska et al., 2010). Cell-based
strategies to remyelinate spared axons is an attractive emerging approach in the treatment of SCI.

NEUROTRANSMITTERS AND ITS RECEPTORS IN SPINAL CORD INJURY

In the adult nervous system, neurotransmitters mediate cellular communication within neuronal circuits. Neurons within the spinal cord represent a primary site for the integration of somatosensory input. Spinal sensory integration is a dynamic process regulated by factors that include multisensory convergence and pathway selection (Lundberg, 1979; Baldissera et al., 1981; Jankowska, 1992). The transmission of the sensory information begins with activation of the peripheral receptors of primary afferent neurons whose cell bodies lie within the dorsal root ganglia (DRG) and whose central terminals project to secondary neurons in the dorsal horn of the spinal cord. Several neurotransmitters and a large variety of receptors have been found in the superficial laminae of the dorsal horn. Transmission of the somatosensory information from the primary afferent fibers to the secondary dorsal horn neurons depends on the balance between the excitatory effects of excitatory amino acids and the inhibitory actions of several other transmitter systems. Neurotransmitter signalling has profound influence on the normal sequence of events involved in development of the spinal cord and hence locomotion. Neurotransmitters that promote cell proliferation include ACh, 5-HT, GABA.

5-HT

5-HT is present in the axons and terminals of raphe-spinal neurons in the dorsal horn, especially in the superficial laminae, laminae I-III. The origin of serotonergic projection to the dorsal horn is mainly the nucleus raphe magnus (Dahlström & Fuxe, 1965; Fuxe, 1965; Basbaum et al., 1978; Miletic et al., 1984). 5-HT and several peptides may be co-localized in the same raphe neurons and in
their terminals. 5-HT may also be co-localized with GABA (Millhorn et al., 1987a,b). Molecular cloning has identified seven distinct families of 5-HT receptors (5-HT1-7). The 5-HT3 family consists of ligand gated ion channel receptors. The other 6 families interact with G-proteins and are coupled to second messengers. Three 5-HT receptor subtypes influence the dorsal horn somatosensory processing: 5-HT1, 5-HT2, and 5-HT3. There are three major sources of 5-HT receptors to the spinal cord dorsal horn: the DRG cells, the intrinsic spinal neurons and the descending systems. Neonatal capsaicin treatment or dorsal rhizotomy decrease 5-HT1A and 5-HT3 receptor binding in laminae I and II, but some still remains, indicating both pre and post synaptic localizations. A large majority of the 5-HT receptors in the dorsal horn do not participate in classic synapses, but are found in extra synaptic sites along the dendrites and somas.

The 5-HT systems are widespread throughout the brain, with most of the cell bodies of serotonergic neurons located in the raphe nuclei of the midline brain stem (Palacios et al., 1990). The largest collections of 5-HT neurons are in the dorsal and median raphe nuclei of the caudal midbrain (Jacobs & Azmitia, 1992). The neurons of these nuclei project widely over the thalamus, hypothalamus, basal ganglia, basal forebrain and the entire neocortex. Interestingly, these 5-HT neurons also provide a dense subependymal plexus throughout the lateral and third ventricles. Activation of this innervations result in 5-HT release into the cerebrospinal fluid (CSF) and measurement of 5-HT content in CSF in disease states will largely reflect this pool (Chan-Palay, 1976).

The activation of 5-HT receptors can produce multiple physiological events, as 5-HT receptor families can either promote or inhibit different second messenger systems. Intrathecally administered 5-HT can either inhibit or stimulate (Hylden & Wilcox, 1983; Clatworthy et al., 1988) nociceptive reflexes. Iontophoretic application in the vicinity of dorsal horn neurons generally causes inhibition (Griersmith & Duggan, 1980), although excitatory effects have also
been reported (Todd & Millar, 1983). It has been suggested that the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes mediate selective inhibition of nociceptive neurons, whereas 5-HT$_{1A}$ agonists facilitate nociceptive responses (El-Yassir et al., 1988; Alhaider & Wilcox, 1993). Spinal 5-HT$_3$ mediated analgesia involves GABA receptors, probably through the excitation of GABAergic interneurons (Alhaider & Wilcox, 1991).

A variety of neuro modulators play various roles in the activation of different neuron types, aiding in locomotion. 5-HT is known to play a facilitory role in locomotor circuitry by increasing motoneuron excitability, modulating spinal CPG (Salles et al., 1979; Sillar et al., 1997; Rossignol et al., 1998) and improving locomotor behaviour following spinal injury (Kim et al., 1999; Ribotta et al., 2000). A small amount of 5-HT can activate super-sensitive motor neurons (Li et al., 2007). The 5-HT system surrounds the corticospinal tract in the lateral funiculus, which explains the correlation between losses of 5-HT and motor deficit after SCI (Shapiro, 1997). Muscle paralysis after SCI is partly caused by a loss of brain stem derived 5-HT, which normally maintains motoneuron excitability by regulating crucial persistent calcium currents (Murray et al., 2010).

5-HT AS CO-MITOGEN

In rats, 5-HT neurons in the brain stem raphe are among the first neurons to differentiate in the brain and play a key role in regulating neurogenesis (Kligman & Marshak, 1985). Lauder and Krebs (1978) reported that para chloro phenyl alanine, a 5-HT synthesis inhibitor, retarded neuronal maturation, while mild stress, a releaser of hormones, accelerated neuronal differentiation. These workers defined differentiation as the cessation of cell division measured by incorporation of $^3$H thymidine. Since then, many other workers have shown a role for 5-HT in neuronal differentiation (Marois & Croll, 1992; Hernandez, 1994). The effects of 5-HT on morphology have long been known. For more than
50 years, 5-HT has been known to constrict blood vessels (Page, 1968) and induce shape changes in skeletal muscle (at both the light and electron microscope level) (O’Steen, 1967), platelets (Leven et al., 1983), endothelial cells (Welles et al., 1985), and fibroblast (Boswell et al., 1992). In the periphery, 5-HT originates largely from mast cells, which can produce, release and reuptake 5-HT. The released 5-HT, then act as a chemotactic, increase vascular permeability, vasodilatation and smooth muscle spasm (Metcalfe et al., 1981). In addition to its role in morphological changes, 5-HT also has been shown to play a role in cell proliferation. In cultured rat pulmonary artery smooth muscle cells, 5-HT induces DNA synthesis and potentiates the mitogenetic effect of platelet-derived growth factor-BB (Eddahibi et al., 1999). 5-HT effects on cell proliferation are involved with phosphorylation of GTPase activating protein an intermediate signal in 5-HT induced mitogenesis of smooth muscle cells (Lee et al., 1997). Earlier studies from our laboratory showed that 5HT acting through specific receptor subtypes 5HT2 (Sudha & Paulose, 1998) control cell proliferation and act as co-mitogens. Previous work also suggests that 5-HT can also trigger the cell division in the dopaminergic neurons in substantia nigra (Nandhu, 2011). Thus, there is evidence that 5-HT is involved in a variety of cellular processes involved in regulating metabolism, proliferation and morphology.

**GABA**

GABA have been localized in the spinal cord dorsal horn (Price et al., 1984; Towers et al., 2000). GABAergic neurons are found throughout the gray matter of the spinal cord, with a higher frequency in the superficial laminae (laminae I-III) (Magoul et al., 1987; Todd & Sullivan, 1990; Powell & Todd, 1992; Spike & Todd, 1992; Todd et al., 1992). It is an inhibitory neurotransmitter in the dorsal horn as they increase Cl- conductance through neuronal cell membranes and hence produce inhibitory postsynaptic potentials. It functions both pre and post synaptically. GABAergic neurons play an important role in spinal
cord function and dysfunction (Coull et al., 2003) and SCI (Craig, 2002; Finnerup & Jensen, 2004). After SCI, a reduction occurs in the number of inhibitory synapses related to GABA.

Three GABA receptor subtypes have been identified: the GABA_\text{A} receptor subtype, which mediates rapid ionotropic transmission; the GABA_\text{B} receptor subtype, which mediates a variety of metabotropic responses; and the GABA_\text{C} subtype, which has not yet been found in the dorsal horn. There is significantly more GABA_\text{B} than GABA_\text{A} receptor ligand binding in the dorsal horn. Both receptor subtypes can be found in great numbers on the primary afferent terminals. There is a heavy concentration of GABA receptors exists in lamina II and much thoughts has therefore been given to the presynaptic modulation of the fine caliber, presumably nociceptive, primary afferent input. GABA_\text{B} receptors are found in abundance in laminae I, III and IV and accordingly are thought to be involved in the presynaptic control of A_\delta and A_\beta primary afferent fibers. Activation of presynaptic GABA_\text{B} receptors decreases glutamate release from primary afferent terminals in the spinal cord (Iyadomi et al., 2000). Loss of GABA leads to neuropathic pain after SCI (Meisner et al., 2010; Young & Claire 2011; Lee et al., 2012)

Almost all cell bodies in the DRG are positively stained with an antibody to the subunits of the GABA_\text{A} receptors. Moreover, intrinsic dorsal horn neurons contribute significantly to the GABA_\text{A} receptor population in the dorsal horn. Baclofen (a GABA agonist), a chlorophenyl derivative of GABA and selective ligand for GABA_\text{B} receptors, depresses both monosynaptic and polysynaptic transmission in the dorsal horn possibly through a decrease in transmitter release rather than by any antagonism at postsynaptic receptors. It has been reported that GABA_\text{B} sites, unlike GABA_\text{A} sites, are present in high concentrations in laminae I, II, III and IV of the dorsal horn and that after the neonatal administration of capsaicin this binding is reduced by 40-50% (Price et al., 1984). It has been
hypothesized that GABA\textsubscript{A} sites regulate presynaptic glutamate release (Ishikawa et al., 2000). GABA\textsubscript{A} receptors induce neuronal changes from hyperpolarization to depolarization and further mediate transmembrane calcium influx (Shulga et al., 2008).

The inputs to the basal ganglia portion of the motor circuit are focused principally on the putamen, whereas the caudate nucleus and the nucleus accumbens are the principal input sites of the limbic circuit depicts a simplified scheme of the 'motor circuit' (Albin et al., 1989). This postulates that in the normal brain there exists a balance between direct inhibitory input (GABA, co-localised with substance P) and indirect excitatory input (aspartate/glutamate) to the medial globus pallidus, which in turn controls thalamocortical activation.

In the GABAergic neurons in the lumbar spinal cord, GABA coexists with glycine or ACh (Rosemary et al., 1993). It is assumed that chronic SCI increases GABA receptor sensitivity in the spinal cord, possibly due to down regulation of the inhibitory GABAergic mechanism (Minoru et al., 2008). GABA administration has been found to cause locomotor hyperactivity. GABA within the CNS is involved in the control of locomotor activity. This makes GABA supplementation more important for the development of therapeutics for SCI.

**GABA AS CO-MITOGEN**

GABA, the most abundant and important inhibitive neurotransmitter in the CNS, plays a regulatory role in regeneration of various nerve cells (Ben-Ari et al., 1989; Baher et al., 1996; Chiba et al., 1997; Behar et al., 2000; Haydar et al., 2000; Luyt et al., 2007). Through embryonic development, GABA was demonstrated as acting as a chemo-attractant and being involved in the regulation of progenitor cell proliferation. For example, GABA induces migration and motility of acutely dissociated embryonic cortical neurons (Baher et al., 1996; Behar et al., 2000). In addition, the neurotransmitters GABA and glutamate
reportedly reduce the number of proliferating cells in dissociated or organotypic cultures of neocortex (LoTurco et al., 1995). In contrast, GABA was shown to promote cell proliferation in cultures of cerebellar progenitors (Fiszman et al., 1999). GABA also dramatically increases proliferation in the ventricular zone of the embryonic cerebrum in organotypic cultures by shortening the cell cycle. However, a reverse effect was observed in the subventricular zone (Haydar et al., 2000). Thus, during embryonic neurogenesis, GABA emerges as an important signal for cell proliferation and migration, but its precise regulation is depend on the region and cell type affected. GABA affects the proliferation of embryonic stem cells (Michael et al., 2008).

Cellular response to GABA is mediated through its known receptors and the intracellular signals associated with them. The contribution of GABA, to both chemo-attraction (Behar et al., 2000) and cell proliferation (Haydar et al., 2000) was indicated. However, in some aspects of cell motility there is an apparent involvement of GABA dependent G protein indicating a role of GABA (Behar et al., 2000). GABA acts as a trophic factor not solely during prenatal neurogenesis but also in the postnatal period in injured tissue. The effect of GABA involves stimulation of cell proliferation and Nerve growth factor (NGF) secretion (Ben-Yaakov & Golan, 2003).

GABA increases synaptic plasticity. Soltani et al., (2011) reported that GABA promotes proliferation of β-cells in pancreas. GABAergic inputs to hippocampal progenitor cells promote neuronal differentiation (Tozuka et al., 2005). There has been evidence that baclofen, GABA agonist increase 5-HT secretion in lumbar spinal tract and rat striatum (Waldmeier & Fehr, 1978). Also, baclofen is seen to act as a potent co-mitogen, triggering DNA synthesis in primary cultures of rat hepatocytes, mediated through the G(i) protein-coupled GABAB receptors (Biju et al., 2002).
ACETYLCHOLINE

ACh, one of the monoamines, is found in the motor nuclei of the cranial nerves and in the motor neurons of the spinal cord. In these areas it serves as the chemical messenger for neuromuscular transmission. ACh is also present in certain ascending pathways within the CNS. Cholinergic neurons project in a widespread ascending system from the medial septal nuclei to the hippocampus and from the nucleus basalis of Meynert to the cerebral cortex. The basal ganglia are rich in this monoamine and enzymes responsible for its synthesis and break down (ChAT and AChE, respectively). Large cholinergic neurons have been found in the human striatum. ACh mediates its facilitatory actions predominantly post-synaptically. The presumed postsynaptic action of ACh is consistent with the dominant postsynaptic localization of cholinergic receptors in the spinal cord dorsal horn (Gillberg & Askmak, 1991). Intrinsic cholinergic innervation has been demonstrated in the spinal cord. The most prominent cholinergic system consists of cholinergic cells associated with cranial nerve nuclei and motoneurons of the spinal cord (Ribeiro-da-Silva & Cuello, 1990; Wets & Vaughn, 1994). ACh exerts its actions through mainly two kinds of receptors: muscarinic and nicotinic receptors.

Preganglionic neurons that originate in the brain stem and sacral spinal cord communicate with postganglionic neurons by extending very long axons that release the neurotransmitter, ACh. The postganglionic neurons have very short axons that release ACh onto the targeted organ to modulate the intrinsic activity of the eye, lacrimal gland, salivary gland, heart, bronchi and lungs, small intestine, stomach, gallbladder, liver, pancreas, large intestine, rectum, genitalia, blood vessels, bladder, legs and hands. Each of these targeted organs expresses ACh receptors to respond to the parasympathetic nervous system.
At the cellular level, cholinergic neurotransmission from motoneurons and interneurons is involved in modulating neuronal excitability in the spinal cord of many vertebrates. Other cholinergic neurons in the mammalian spinal cord include central canal cells (lamina X) and the partition cells, the latter of which extend from the central canal to the lateral edge of the gray matter and have been proposed to participate in locomotor activity on the basis of c-fos staining and electrophysiological recordings (Barber et al., 1984; Borges & Iversen, 1986; Sherriff & Henderson, 1994; Carr et al., 1995; Huang et al., 2000). Recently, evidence has come forth that medial partition cells give rise to the large cholinergic C terminals on motoneurons (Miles et al., 2007). Activation of muscarinic cholinergic receptors on motoneurons increases excitability through reduction of the after-spike hyperpolarization (Chevallier et al., 2006; Miles et al., 2007). In addition to motoneurons, many other spinal neurons of the dorsal and ventral horns and lamina X are responsive to ACh (Ziegla¨nsberger & Reiter, 1974; Jiang & Dun, 1986; Urban et al., 1989; Bordey et al., 1996a,b) indicating that both motoneuron and interneuron excitability are modulated by ACh.

ACh was found to exert powerful modulation of locomotor network activity, cellular properties and synaptic strength in the spinal cord of lamprey (Katharina et al., 2008). It has been reported that cholinergic modulation of the lamprey spinal locomotor network is likely produced by both motoneurons and cholinergic interneurons acting via combined postsynaptic and presynaptic actions (Katharina et al., 2008, Quinlan & Buchanan, 2008). The CPG for swimming in the lamprey spinal cord has provided a model system to study the vertebrate locomotor network. During fictive swimming, sufficient ACh is present in the isolated spinal cord to provide ongoing modulation of the locomotor network through both nicotinic and muscarinic receptors (Quinlan et al., 2004).

In hatchling Xenopus, cholinergic feedback from spinal motoneurons provides excitation to both motoneurons and interneurons of the locomotor
network and cholinergic excitation during locomotion in Xenopus embryos constitutes a significant portion of the depolarizing drive to these neurons (Perrins & Roberts, 1995a,b,c). In other organisms, such as turtles, salamander and neonatal rats and mice, cholinergic input contributes to excitatory drive during locomotion, inducing or promoting the induction of rhythmic activity (Cowley & Schmitt, 1994; Perrins & Roberts, 1995 a,b,c; Kiehn et al., 1996; Zhao & Roberts, 1998; Guertin & Hounsgaard, 1999; Myers et al., 2005; Carlin et al., 2006; Chevallier et al., 2006; Miles et al., 2007), although cholinergic effects on network activity are diverse.

Evidence suggesting that ACh function as a synaptic transmitter in spinal cord has come from biochemical studies demonstrating its presence in the spinal cord (Barnes & Worrall, 1968) and from the evidence that stimulation of appropriate spinal structures causes the release of ACh (Kuno & Rudomin, 1966). ACh plays a role in morphogenic cell movements, cell proliferation, growth and differentiation. ACh released from growing axons regulates growth, differentiation and plasticity of developing CNS neurons. Growth promoting actions of endogenous ACh are evident from the severe growth defects observed in ChAT deficient nematode and drosophila mutant (Rand & Russel, 1984). ACh regulates neurite outgrowth (Lopton & Kater, 1989). Cholinergic neurons appear earliest in the rat spinal cord and brain at embryonic stage (Semba, 1992). ACh also modulates spinal sensory processing in the dorsal horn (Myslinski & Randic, 1977; Urban et al., 1989). As it appears that there are no descending cholinergic systems in the rat (Bowker et al., 1983; Willis & Coggeshall, 1991), these actions probably arise from a population of intrinsic cholinergic interneurons found in the dorsal horn (Barber et al., 1984; Todd, 1991). Coronas, et al., (2000) found that ACh neuritic outgrowth in the rat olfactory bulb.

Neuromodulation through cholinergic receptors is widespread in the CNS and the effects of ACh on the neural network that generates locomotor like
activity in the spinal cord are diverse. Starting with its proper development, the spinal locomotor network depends on cholinergic transmission (Hanson & Landmesser, 2003; Myers et al., 2005). During drug-induced locomotor activity in neonatal rat and mouse, exposure of the isolated spinal cord to ACh or cholinergic agents alters the amplitude and frequency of locomotor activity (Myers et al., 2005; Miles et al., 2007) and exposure to ACh alone can induce rhythmic activity in the isolated cord (Cowley & Schmidt, 1994). ACh, carbachol (a mixed muscarinic/nicotinic agonist) and nornicotine (a nicotine metabolite with agonist properties) also had neuroprotective properties in striatal cultures (Marin et al., 1994).

It is reported that functional activity of grafted spinal cord transforms into motor neurons (Demierre et al., 1990; Sieradzan & Vrbová, 1991; Clowry & Vrbová, 1992) and it can be evaluated by ACh release (Rosario et al., 2007). ACh levels in the ventral spinal cord are likely to reflect motoneuronal activity. Cholinergic interneurons in the lumbar spinal cord are involved in the production of fictive locomotion (Huang et al., 2000). Cholinergic inputs from the mesencephalic locomotor region to reticulospinal cells play a substantial role in the initiation and the control of locomotion (Le ray et al., 2003). Since ACh serve as excitatory neurotransmitter within the spinal cord, it could contribute to the functional neurologic impairment that follows injury (Faden et al., 1986). Cholinergic receptor density is affected in SCI (Jay, 2002).

MUSCARINIC RECEPTORS

Dale (1914) is the first one to divide the actions of ACh into nicotinic and muscarinic. Muscarinic receptors have been demonstrated to be present in spinal cord of human, rat and cat using invitro autoradiography with a variety of tritiated ligands, including [3H]-QNB (Kayaalp & Neff, 1980; Yamamura et al., 1983; Gillberg et al., 1984; Scatton et al., 1984; Seybold & Elde, 1984; Villiger & Faull,
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Muscarinic receptors have also been detected by radioligand autoradiography in the prenatal rat brain (Lichtensteiger, 1988). They are expressed two to three times higher than nicotinic acetyl choline receptor (nAChR) in the spinal cord (Gillberg et al., 1988).

The muscarinic class of ACh receptors are widely distributed throughout the body and serve numerous vital functions in both the brain and autonomic nervous system (Lefkowitz et al., 1990). Within the nervous system, muscarinic receptors are present on some axon endings (heteroreceptors and autoreceptors), regulating neurotransmitter release (Raiteri et al., 1984; Akaike et al., 1988; Vizi et al., 1989; Raiteri et al., 1990). These receptors are also on the soma and dendrites of many types of neurons, including cholinergic and noncholinergic neurons (Wamsley et al., 1984; Raiteri et al., 1990).

Muscarinic ACh receptors are known to regulate numerous fundamental physiological processes, including central sensory, vegetative and motor functions (Wess et al., 1990; Levin et al., 1995; Brown et al., 1996; Levin et al., 1997). It is reported that muscarinic cholinergic effects of ACh are important in the normal function of both the sensory and motor systems (James et al., 1981). Muscarine stimulated neural activity lead to locomotion. Activation of spinal muscarinic receptors increases the intraspinal release of ACh and that inhibition of these receptors decreases ACh release (Höglund et al., 2000). The stimulation of neuronal muscarinic receptors induce neurite outgrowth in chick dorsal root ganglia, neuroblastoma cells and in a rat pheochromocytoma neuronal cell line transfected with the muscarinic M1 receptor (Kathryn et al., 2009).

Activation of muscarinic receptors in the periphery causes a decrease in heart rate, a relaxation of blood vessels, a constriction in the airways of the lung,
an increase in the secretions and motility of the various organs of the gastrointestinal tract, an increase in the secretions of the lacrimal and sweat glands, and a constriction in the iris sphincter and ciliary muscles of the eye. In the brain, muscarinic receptors participate in many important functions such as learning, memory and the control of posture. Muscarinic receptors indirectly stimulate GABA release. For instance, Urban et al., (1989) reported an increase in excitability of spinal cord dorsal horn neurons by ACh, while Baba et al., (1998) reported a muscarinic induced facilitation of GABA release in substantia gelatinosa neurons of the rat.

mRNAs encoding five genetically distinct muscarinic ACh receptors are present in the CNS (Kubo et al., 1986; Bonner et al., 1987). Because of their pharmacological similarities, it has not been possible to detect the individual encoded proteins; thus, their physiological functions are not well defined. These receptor subtypes had marked differences in regional and cellular localization as shown by immunocytochemistry. By convention, molecularly identified subtypes are referred to as m1 to m5, and pharmacologically identified subtypes are designated M1 to M4 (Birdsall et al., 1989). The M1 to M4 subtypes generally correspond to the m1 to m4 subtypes (Waelbroeck et al., 1990; Caulfield, 1993). Wamsley and colleagues (1984) showed that high and low affinity muscarinic binding sites are present in spinal cord. Villiger and Faull (1985) named these receptors M1 and M2, respectively.

Studies suggest that M2 binding sites were distributed throughout the dorsal and ventral horns, whereas M3 binding sites were localized to laminae I to III of the dorsal horn. Only background levels of M1 binding sites were detected. The finding that M2 and M3 binding sites were localized to the superficial laminae of the dorsal horn where nociceptive Ad and C fibers terminate which suggest the possibility that either or both of these muscarinic receptor subtypes modulate anti nociception. The present demonstration of M4 binding sites in
spinal cord is consistent with the possibility that M2 and/or M4 receptors are involved in the regulation of blood pressure at the spinal level (Hoglund & Baghdoyan, 1997).

In particular, muscarinic M1, M3 and M4 subtypes are abundantly and broadly detected in rat brain, including the cerebral cortex, striatum and hippocampus. Next in abundance is mRNA for the m2 receptor. The muscarinic M1 protein is present in cortex and striatum and was localized to cell bodies and neurites, consistent with its role as a major postsynaptic muscarinic receptor. The muscarinic M2 receptor protein is abundant in basal forebrain, scattered striatal neurons, mesopontine tegmentum and cranial motor nuclei; this distribution is similar to that of cholinergic neurons and suggests that muscarinic M2 is an autoreceptor. However, muscarinic M2 receptor was also present in noncholinergic cortical and subcortical structures, providing evidence that this subtype presynaptically modulate release of other neurotransmitters and/or function postsynaptically. Heart is a rich source for the muscarinic M2 receptor and exocrine glands. The abundance of muscarinic M3 mRNA was greatest in the cerebral cortex and hippocampus, but low in the caudate putamen and in caudal regions of the brain. Smooth muscles are rich in the muscarinic M3 subtype. The muscarinic M4 receptor was enriched in neostriatum, olfactory tubercle and islands of Calleja, indicating an important role in extrapyramidal function. The muscarinic M5 receptor message is least abundant in brain (Liao et al., 1989; Vilaro et al., 1990).

All five muscarinic receptors are homologous proteins consisting of between 460 and 590 amino acids for the human receptors (Peralta et al., 1987; Bonner et al., 1988). There is a very high degree of homology at the amino acid level for each receptor across species (for example, human m1 has 98.9% identity with porcine m1) (Peralta et al., 1987). Analysis of the primary sequences of the muscarinic subtypes shows that these receptors are members of a superfamily of
genes including the opsins and numerous receptors which signal through G proteins (Kubo et al., 1986; Bonner et al., 1987). These receptors are typified by the presence of seven hydrophobic regions in their sequence which form alpha helixes that span the membrane. The transmembrane segments of the muscarinic receptor represent the regions of highest homology among the different subtypes and across other members of this large family of G-protein-linked receptors. If the sequences of the five subtypes of the muscarinic receptor are aligned to achieve maximum identity, it can be seen that differences in the lengths of the sequences arise from differences in the extracellular amino terminis, the cytoplasmic carboxy terminis and the third intracellular loop. The remaining portions of the protein—namely, the seven transmembrane segments, the three extracellular loops and the first two cytoplasmic loops are all the same length. There is 63% identity among the amino acids of seven transmembrane segments of the human M1–M5 subtypes and most of the remaining residues in these segments are conservative replacements. The greatest divergence arises from the third cytoplasmic loop. This loop varies in length from 156 (M1) to 239 (M3) residues in the five human sequences and it accounts for 34–45% of the total number of amino acids. A comparison of the sequences shows that the muscarinic M1, M3 and M5 subtypes show maximum homology with each other, whereas the muscarinic M2 and M4 subtypes constitute a separate homologous group.

MUSCARINIC RECEPTORS IN SPINAL CORD INJURY AND REGENERATION

The cholinergic system is important for regulation of neuronal activity and body movement (Franchi, 2000; Lloyd & Williams, 2000; Sun et al., 2002). 80% or more of muscarinic binding sites in the spinal cord cell cultures are on neuron (Brookes & Burt 1990). The muscarinic receptors mediate increment of intracellular calcium concentrations following nerve injury which suggests that the cholinergic system is associated with nerve regeneration and repair following
injury (Dawei et al., 2010). Intracellular free calcium levels are closely related to regeneration in the rat hippocampus and spinal cord cortical neurons (Sahly et al., 2006; Shulga et al., 2008; Kamber et al., 2009). Neuronal damage can lead to a transient increase of free calcium ion levels at the injury site, thereby increasing neuronal regeneration and repair (Kamber et al., 2009). Similarly, increased levels of calcium ions in hippocampal neurons are necessary for repair of injured central neurons (Shulga et al., 2008). ACh induced increase of calcium ion levels has been shown following muscarinic M1 and/or M3 cholinergic receptor mediated release of intracellular calcium in a retinal regeneration study (Ohmasa & Saito, 2003). M2 and M4 receptors are expressed during retinal regeneration which suggests their role in neuronal regeneration (Cheon et al., 2001).

Traumatic SCI in rats and ischemic SCI in rabbits are associated with localized decreases in muscarinic receptor binding (Faden et al., 1986). The spinal cholinergic system and muscarinic receptors are important in nociception. Intrathecal administration of cholinergic muscarinic agonists or AChE inhibitors produces analgesia in both animals and humans (Iwamoto & Marion, 1993; Naguib & Yaksh, 1994; Hood et al., 1997). Importantly, the muscarinic M2 subtype is the predominant muscarinic receptor in the spinal cord dorsal horn (Hoglund & Baghdoyan, 1997; Yung & Lo, 1997). Studies performed in muscarinic receptor knockout mice provide further evidence that the muscarinic M2 receptors play an essential role in cholinergic analgesia (Gomeza et al., 1999).

NICOTINIC ACETYL CHOLINE RECEPTORS

nAChRs receptors are characterised through their interaction with nicotine in tobacco. The binding of nicotine can activate nAChRs, modifying the neurons in two ways: the depolarisation of the membrane through the movement of cations results in an excitation of the neuron, while the influx of calcium acts through intracellular cascades affect the regulation of certain genes and the release of neurotransmitters.
Radioactive nAChR ligands preferentially bind to the substantia gelatinosa, laminae III or IV, with the emphasis on laminae III and IV (Wamsley et al., 1981a; Gillberg & Aquilonius, 1985; Gillberg & Wiksten, 1986). It is not clear whether nAChRs are restricted to any particular cell type or sensory modality. Both nicotinic and muscarinic ligands bind to DRG cells and dorsal rhizotomy significantly reduces their binding sites in the dorsal horn (Wamsley et al., 1981a,b; Seybold & Elde, 1984; Seybold, 1985).

Signal transduction is relatively simple at nAChRs. The nAChRs consisting of five subunits surrounding an internal channel is its own ligand-gated ion channel (Popot et al., 1976; Conti-Tronconi et al., 1982). The binding of ACh to nAChRs brings about their activation. When two molecules of ACh bind a nAChR, a conformational change occurs in the receptor, resulting in the formation of an ion pore. At the neuromuscular junction, the opening of a pore produces a rapid increase in the cellular permeability of sodium and calcium ions, resulting in the depolarisation and excitation of the muscle cell, thereby producing a muscular contraction. The activation of neuronal nAChRs also causes the movement of cations through the opening of an ion channel, with the influx of calcium ions affecting the release of neurotransmitters. nAChRs on a postganglionic neuron are responsible for the initial fast depolarisation of that neuron.

nAChRs are composed of five types of subunits: alpha (α1-α10), beta (β2-β5), delta, epsilon and gamma. These receptors span the membrane, containing extracellular, transmembrane and cytoplasmic domains, the latter being the most variable. nAChRs are always pentamers, with the subunits arranged symmetrically around a central receptor channel. The receptors always contain two or more alpha subunits, which are critical in ACh binding. The ACh binding site is comprised of a dimer formed by the α subunits (principal component) plus an adjacent subunit (complementary component), where binding to both sites is required for the channel to open.
nAChRs are involved in a wide range of physiological processes and can be either neuronal or muscle type. Muscle type nAChRs are localised at neuromuscular junctions, where an electrical impulse from a neuron to a muscle cell signals contraction and is responsible for muscle tone; as such, these receptors are targets for muscle relaxants. The many types of neuronal nAChRs are located at synapses between neurons, such as in the CNS where they are involved in cognitive function, learning and memory, arousal, reward, motor control and analgesia.

nAChRs appear to have a predominant presynaptic location and facilitate the release of a number of different neurotransmitters and hormones including ACh, GABA, glutamate, dopamine, 5-HT and norepinephrine (O’neill et al., 2002). Increased expression of calcium binding proteins by nicotine could protect the cells from a number of insults that cause cellular damage secondary to increases in cytoplasmic calcium overload (Prendergast et al., 2001, Freir & Herron, 2003). nAChR mediate communication across synapses.

In mammals, nAChR play a crucial role in motor control (Marc et al., 1991). Following peripheral nerve injury, the expression of numerous receptors involved in nociceptive processing is altered in the superficial dorsal horn of the spinal cord. Activation of nAChR promotes survival of chicken spinal motoneurons that would otherwise undergo apoptosis when deprived of trophic factors (Messi et al., 1997). It is suggested that spinal ACh release is regulated by different nAChR ACh receptors. These receptors tonically regulate spinal ACh release either directly or indirectly via inhibitory interneurons. Peripheral nerve injury produces a variety of changes within the spinal cord both ipsilaterally and contralaterally, including changes in the expression of nAChRs (Yang et al., 2004).
Abundant evidence for the presence of nAChRs in the spinal cord, particularly in the superficial laminae of the dorsal horn where nociceptive Ad and C fibres terminate (Gillberg et al., 1988; Khan et al., 1994b; Roberts et al., 1995; Khan et al., 1996; Khan et al., 1997; Marubio et al., 1999) and in other parts of the nociceptive system (Adem et al., 1989; Iwamoto, 1991; Bitner et al., 1998; Flores, 1998; Ryan & Loiacono, 2000). In the spinal cord, nAChRs are expressed on primary afferents (Roberts et al., 1995; Li et al., 1998; Genzen & McGehee, 2003; Miao et al., 2004; Khan et al., 2004), descending noradrenergic (Li et al., 2000) and serotonergic (Cordero-Erausquin & Changeux, 2001) fibers presynaptically, as well as postsynaptically on spinal inhibitory and excitatory neurons (Bradaia & Trouslard, 2002a,b; Cordero-Erausquin et al., 2004; Genzen & McGehee, 2005). Previous studies suggest that the α4β2 and α7 nAChRs on primary afferent C-fibers are likely responsible for the nociceptive responses while an α3β4 or a previously undescribed nAChRs are responsible for the antinociceptive properties (Rueter et al., 2000; Khan et al., 2001).

nAChR have been detected by radioligand autoradiography in the prenatal rat brain (Lichtensteiger, 1988; Schlumpf, 1991). The two most prominent nAChRs expressed in the mammalian CNS are those consisting of either α4/β2 subunits or homomeric α7 receptors (Marks et al., 1986; Lukas et al., 1999). The brain alpha-bungarotoxin receptor is a homomeric receptor consisting of only α7 nAChR subunits (Hogg et al., 2003). The α7 receptor is unique because of its high permeability to calcium. Numerous studies have shown that α7 receptors are involved with cognition, synaptic plasticity and presynaptic neurotransmitter release and regulation of immune function (Kem, 2000). The cellular mechanisms involved with nicotine-induced neuroprotection against excitotoxins are not well established, but previous studies have pointed to up regulation of intracellular calcium binding proteins, (Prendergast et al., 2001; Stevens et al., 2003) changes in nitric oxide mediated signaling (Shimohama et al., 1993) and other signaling
events downstream from nAChR activation (O’neill et al., 2002; Dajas & Wonnacott, 2004).

Cholinergic stimulation of the α7 homo pentameric nAChR inhibits production of pro-inflammatory cytokines (Bernik et al., 2002; Borovikova et al., 2000; Wang et al., 2003; Saeed et al., 2005; Nizri et al., 2006), expression of endothelial cell adhesion molecules and leukocyte recruitment during inflammation (Saeed et al., 2005). The anti-inflammatory effect of α7 nAChR stimulation is partly related to the regulation of cytokine production by macrophages (Borovikova et al., 2000; Bernik et al., 2002; Wang et al., 2003; Shytle et al., 2004; Saeed et al., 2005; Ulloa, 2005; RosasBallina et al., 2009) and T lymphocytes (Nizri et al., 2006). The α7 nAChR subunit is considered a regulator of macrophage/monocyte activation for the release of molecules (TNFα, Inter leukine (IL-1β)) involved in inflammatory responses and apoptotic pathways (Wang et al., 2003; Pavlov & Tracey, 2005; Ulloa, 2005; Yoshikawa et al., 2006; Tracey, 2007).

NICOTINIC RECEPTORS IN SPINAL CORD INURY AND REGENERATION

nAChR agonists and antagonists have been shown to be neuroprotective in a variety of in vivo and in vitro experimental models (O’neill et al., 2002; Dajas & Wonnacott, 2004). There is solid evidence for involvement of both α4/β 2 and α7 nAChRs in nicotine-mediated neuroprotection (Prendergast et al., 2001; O’neill et al., 2002; Dajas & Wonnacott, 2004). Nicotine has been shown to reduce apoptotic cell death in a wide variety of model systems including neurons, lung cancer cell lines and the cardiovascular system (Garrison et al., 2001; Opanashuk et al., 2001; Suzuki et al., 2003; Weilgus et al., 2004). Audesirk and Cabell (1999) reported that nicotine not only did not influence neuronal survival or neurite production but increases the branching of both axons and dendrites.
nAChR are up regulated during optic nerve regeneration (Hieber et al., 1992). Spinal nerve ligation increases the numbers of cells expressing the α3 subunit and the number of fibers expressing the α5 subunit (Vincler & Eisenach, 2004). The survival of newborn neurons can be controlled by the activation of β2-containing nAChR (Naguib et al., 2004). α7 nAChRs regulate proliferation of neurons in the hippocampus (Naguib et al., 2004). α7 subunit is reported to have an important role in motor control (Villégie et al., 2010).

**CHOLINERGIC ENZYMES – ChAT & AChE**

Preganglionic autonomic neuron and somatic motoneurons are cholinergic and make up a significant proportion of the total neuronal pool. ChAT, the marker enzyme for cholinergic neurons which synthesizes ACh, is abundant in the spinal dorsal horn, especially in the superficial laminae (Kása & Morris, 1972; Kimura et al., 1981; Barber et al., 1984; Kása, 1986; Phelps et al., 1988; Houser, 1990; Ribeiro-da-Silva & Cuello, 1990; Todd, 1991). ChAT has not been found in DRG cells or in their axons (Barber et al., 1984; Borges & Iversen, 1986). AChE, the enzyme which removes ACh by hydrolysis, has also been localized in the dorsal horn, with highest concentrations in laminae III (Kása, 1986). The anatomical organization of cholinergic systems has been extensively studied by mapping the distribution of AChE using AChE histochemistry and by immunohistochemical staining for the ChAT. ChAT and AChE are cholinergic markers.

The enhancement of spinal cord ChAT activity by K-252a and staurosporine defines a new neurotrophic activity for these small organic molecules and raises the possibility that they activate some regulatory elements in common with the ciliary neurotrophic factor and leukemia inhibitory factor family of neurotrophic proteins. It is known that neuronal depolarization can influence ChAT activity levels and therefore ACh production. Neurons treated with depolarizing agents or stimulated directly by electric current exhibit an increase in
ChAT enzyme activity (Ishida & Deguchi, 1983). Nishi and Berg (1981) reported that a high KCl concentration enhanced ChAT activity, protein synthesis, and other activities in neurons cultured from chick ciliary ganglion. These same phenomena were observed in mouse spinal cord (Ishida & Deguchi, 1983).

In addition to its role in cholinergic neurotransmission, AChE has been implicated in several non-cholinergic actions such as cell proliferation (Appleyard, 1994) neurite outgrowth (Chacón et al., 2003) and haematopoiesis (Silman & Sussman, 2005). Interestingly, AChE responds to various insults including oxidative stress, an important event that has been related to the pathogenesis and progression of a variety of CNS disorders, such as stroke (Ozkul et al., 2007), Alzheimer's diseases (Chauhan & Chauhan, 2006) and diabetes mellitus (Kuhad et al., 2008). Acute stress is known to induce expression of the AChE gene and to increase brain AChE activity (Kaufer et al., 1998). In transgenic mice, overexpression of human AChE is accompanied by progressive cognitive deterioration (Beeri et al., 1995). Decreases in AChE and ChAT have been reported in axotomised cholinergic neurons (Liberman, 1971; Ducker, 1985). AChE activity in cerebral cortex is mainly due to expression of this enzyme in cholinergic neurons and their axons. Neurobehavioural deficit can be caused by increase in cerebellum and cortex AChE activity (Mohamed et al., 2001). Takada (2003) reported that the AChE inhibitor, donepezil, was neuroprotective in cultured cortical neurons exposed to excitotoxins.

**SIGNAL TRANSDUCTION THROUGH SECOND MESSENGERS**

The term signal transduction refers to the mechanism used by the first messenger (the neurotransmitter, neuromodulator, or hormone) of the transmitting cell to convert its information into a second messenger within the receiving cell. Signal transduction will involve a receptor for the first messenger and involve both transducers and effectors. In the field of receptors, a transducer is defined as
a molecule that translates one form of "energy" (e.g., the neurotransmitter) into another form, the second messenger. Effector is a molecule that mediates a specific effect (e.g., an ion channel). Prominent second messengers in brain include cAMP, cGMP and IP3. Altered levels of second messengers mediate the actions of neurotransmitter-receptor activation on some types of ion channels, as well as on numerous other physiological responses.

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

IP3 is a molecule that functions to transfer a chemical signal received by the cell, such as from a hormone, neurotransmitters, growth factors and hypertrophic stimuli such as angiotensin-II, β adrenergic receptor agonists, and Endothelin-1 to various signaling networks within the cell. Two essential signaling pathways are involved in the intracellular generation of IP3. The first signaling pathway is initiated by PLC. PLC are soluble proteins that are partly cytosolic and partly associated with membrane. When a ligand binds to a G protein-coupled receptor that is coupled to a Gq heterotrimeric G protein, the α-subunit of Gq can bind to and induce activity in the PLC isozyme PLC-β, which results in the cleavage of PIP2 into IP3 and Diacylglycerol (DAG) (Biaggioni et al., 2011). IP3 diffuses to the endoplasmic reticulum, where it triggers release of Ca2+ ions into the cytosol. Subsequently, the released Ca2+ and DAG activate protein kinase C. The second signaling pathway involving IP3 generation is initiated by Phosphoinositide 3-Kinase, an enzyme that phosphorylates inositol lipids generating two signaling molecules, PIP2 (Phosphatidylinositol 3,4-Bisphosphate) and PIP3 (Phosphatidylinositol 3,4,5-Trisphosphate). PIP2 and PIP3 function as activators of protein kinases and may regulate G proteins.

There is a very close association between the activation of muscarinic receptors and the formation of IP3 and cGMP. The two events are linked to one another and that one (cGMP response) could be dependent on the other (IP3).
Literature Review

However, with rat brain tissue, there is evidence to suggest that different subtypes of the muscarinic receptor mediate these responses independently (Kendall, 1986; Tonnaer et al., 1991). M1, M3, and M5 subtypes stimulate phosphoinositide hydrolysis. This results in the release of $\text{ca}^{2+}$ ions (Power & Sah, 2000). The rise in calcium regulates a number of process including synaptic plasticity (Bardo et al., 2006; Rose & Konnerth, 2000) that occur in dendritic spines.

In the nervous system, IP$_3$ serves as a second messenger, with the cerebellum containing the highest concentration of IP$_3$ receptors (Worley et al., 1989). There is evidence that IP$_3$ receptors play an important role in the induction of plasticity in cerebellar Purkinje cells (Sarkisov & Wang, 2008). IP$_3$, generated from PIP$_2$ has a vital role in the control of cellular and physiological processes as diverse as cell division, cell proliferation, apoptosis, fertilization, development, behaviour, memory and learning.

3'-5'-cyclic guanosine monophosphate

cGMP formation mediated by ACh was first reported over two decades ago with rat heart (George et al., 1970). Shortly thereafter, muscarinic responses in other tissues were reported. The dependence of the response on $\text{ca}^{2+}$ was established early (Schultz et al., 1973). All the major target organs of parasympathetic cholinergic fibers contain muscarinic receptors that mediate an increase in cGMP (Goldberg & Haddox, 1973). Muscarinic receptors in sympathetic ganglia and in brain also mediate cGMP synthesis. The role of G proteins in muscarinic receptor-mediated cGMP synthesis has not been defined. However, it is synthesis of Nitric oxide and subsequently, cGMP following receptor activation is secondary to the increase in intracellular $\text{ca}^{2+}$, resulting from the release of IP$_3$ from PIP$_2$ by the action of PLC.
3'-5'-cyclic adenosine monophosphate

Neurotransmitters stimulate or inhibit proliferation by activating receptors coupled to different G-proteins and second messenger pathways (Lauder, 1993). It has also been noted that muscarinic M1, M3 and M5 receptors stimulate cAMP accumulation in intact cells (Peralta et al., 1988; Lai et al., 1992). However, this response is downstream from the phosphoinositide response, resulting from calcium or protein kinase C activation of adenylate cyclase. The discovery that the β γ subunits of heterotrimeric G proteins activate the type II and IV adenylate cyclases (Gao et al., 1991; Tang & Gilman, 1991) provides another mechanism for muscarinic enhancement of adenylate cyclase activity that could be demonstrable in a broken cell preparation. This mechanism is dependent upon simultaneous activation by the α subunit of Gs (stimulatory guanine-nucleotide-binding protein) and represent the mechanism by which muscarinic M4 receptors stimulate adenylate cyclase activity in homogenates of the olfactory tubercle (MC, Onali P, 1991).

Phospho Lipase C

Activation of PLC leads to a cascade of events. It results in the breakdown of PIP$_2$ into DAG and IP$_3$ (Fisher, 1987; Berridge, 1983). Stimulation of muscarinic receptors causes DNA synthesis. These mitogenic responses are correlated with increased activity of PLC (Ashkenazi et al., 1989; Gutkind et al., 1991; Mckenzie et al., 1992). Activation of Gq coupled muscarinic M1, M3 and M5 mAChR receptor subtypes stimulates mitogen activated protein kinase C by PLC dependent and PLC independent mechanisms (Wotta et al., 1998). Muscarinic receptor dependent activation of PLC has been reported (Larocca et al., 1994). Receptor-mediated activation of PLC is by no means exclusive to muscarinic subtypes m1, m3 and m5 or to muscarinic receptors in general. The odd numbered muscarinic receptors are most efficiently coupled to this response.
and give the most robust responses, compared to those of the even-numbered receptors (Peralta et al., 1988; Ashkenazi et al., 1989).

**cAMP regulatory element binding protein**

CREB is a plasticity-associated transcription factor, mediating responses to various neurotransmitters, mitogenic factors and differentiating factors (Harris, 2002). Calcium ions act as second messenger in CNS (Clapham, 1995). Extracellular signals can increase intracellular calcium and activates signal transduction pathways. CREB is a critical mediator for calcium dependent gene expression (Sheng et al., 1990). CREB becomes phosphorylated during some forms of synaptic activity (Deisseroth et al., 1996) and is required for several learning processes and adaptive responses in the brain (Bourtchuladze et al., 1994; Maldonado et al., 1996). CREB is involved in glial cell fate determination (Bayatti & Engele, 2001; Harris, 2002). CREB promotes proliferation and survival of neurons and glia in the injured brain (Ong et al., 2000) and mediates cell viability during early embryonic development (Bleckmann et al., 2002). However, in smooth muscle cells, CREB activation (by Ser-133 phosphorylation) associates with suppressed expression of multiple cell cycle regulatory genes and reduced proliferation (Bleckmann et al., 2002; Harris, 2002). Thus, CREB operate either as an inducer or as a suppressor of gene expression, depending on the signal pathway promoting its activation. CREB production up regulates muscarinic M1 receptor (Hu et al., 2010)

CREB is involved in many functions in the nervous system, including neurogenesis and neuronal survival, development, differentiation, neuroprotection, axonal outgrowth and regeneration, synaptic plasticity (Mioduszewska et al., 2003; Persengiev & Green, 2003; Dragunow, 2004; Barco & Kandel, 2006). Genes whose transcription is regulated by CREB include: c-fos, BDNF, tyrosine
hydroxylase and neuropeptides such as somatostatin, enkephalin and corticotropin-releasing hormone (Lauren, 2005).

CREB is a downstream target of cAMP signaling. Multiple lines of evidence define a role for CREB in proliferation and differentiation of certain cells and tissues (Heasley et al., 1991; Spaulding, 1993; Iyengar, 1996). Disruption of CREB activity, using expression of a dominant-negative CREB slows neurite outgrowth and blocks adipocyte differentiation (Engelman et al., 1998; Shimomura et al., 1998).

**APOPTOSIS & SPINAL CORD INJURY**

Apoptosis is a morphologically defined form of programmed cell death. It is a vital component of normal cellular differentiation, development and tissue homeostasis (Wyllie et al., 1980; Ellis et al., 1991; Raff, 1992). Apoptosis is also a key mechanism for removal of damaged, infected or mutated cells that would otherwise present a risk to the organism. Characteristic features of apoptosis include surface membrane blebbing, dilation of the endoplasmic reticulum, externalization of phosphatidylserine at the cell surface, nuclear and cytoplasmatic condensation and DNA fragmentation.

Apoptosis is seen after ischemic or traumatic injury to the CNS (Li et al., 1996; Johnson et al., 1997; Rink et al., 1997), suggesting that active cell death as well as passive necrosis may mediate damage after CNS injury. Both secondary degeneration at the site of SCI and the chronic demyelination of tracts away from the injury appear to be due in part to apoptosis. At the original site of injury, apoptosis occurs about eight hours after the injury in glial cells. A second wave of apoptosis comes about seven days after the injury in the oligodendrocytes of the white matter and the effect is much broader, expanding far away from the original location of the injury.
SCI leads to multifaceted cellular and molecular interactions within the CNS in an effort to repair the initial tissue damage (Thuret et al., 2006). The pathophysiology of SCI is marked by cell death, immune cell transmigration, shearing of cell membranes and axons, disruption of the blood-spinal cord barrier and myelin degradation (Dumont et al., 2001). SCI involves an initial mechanical or primary injury which is then followed by a series of cellular and molecular secondary events resulting in secondary injury that augment the extent of the initial damage and results in the progressive devastation of spinal cord tissue. Secondary injury mediated by multiple injury processes including inflammation, free radical induced cell death and gliosis. Secondary insult immediately after injury is marked by destruction of neuronal and glial cells. It also results in permanent motor and sensory deficits (Taoka & Okajima, 1998; Hagg & Oudega, 2006). Inflammation as a result of secondary injury results in apoptosis of neurons and oligodendrocytes as well as in scar formation and finally in the reduction of neuronal function (Shen et al., 2009). Damage to the spinal cord result in extensive proliferation of microglia and macrophages in and around the injury epicenter. After SCI, cells die by post-traumatic necrosis or by apoptosis (Byrnes et al., 2007). Apoptosis, dependent on active protein synthesis contributes to the neuronal and glial cell death, as well as to the neurological dysfunction, induced by mild-to-moderate severity traumatic insults to the rat spinal cord (Liu et al., 1997). Apoptosis of neurons and oligodendrocytes result in paralysis of patients with SCI (Mizuno, 1998; Mattson, 2000). There are several proteins involved in apoptosis.

**CASPASES**

Apoptosis begins with the activation of a family of proteins known as caspases. Caspases break down normal cellular substrates that are used for such functions as cytoskeleton formation and DNA repair. There are different types of caspases. The caspase-8 is a key enzyme at the top of the apoptotic cascade, both
involved in the extrinsic or death receptors pathway and in the intrinsic mitochondrial pathway. Caspase-8 also known as MACH, FLICE and Mecl5 is synthesized as an inactive single polypeptide chain zymogen procaspase. At least three different mechanisms exist for caspase activation in mammalian cells: recruitment activation, in which a type 1 procaspase is sequestered into an oligomeric activating complex by way of interactions through its extensive prodomain; transactivation, in which the caspase is activated by another caspase and auto activation, in which a caspase initiates its own activating cleavage (Nicholson, 1991). Upon activation by proteolytic cleavage, effector caspases cleave their substrates and inactivate proteins essential for survival, leading to the disintegration of cells (Hengartner, 2000). Knockout data indicate that caspase-8 is required for killing induced by the death receptors Fas, TNF-R1 (TNF receptor 1) and death receptor 3 (Juo et al., 1998; Varfolomeev et al., 1998).

Caspase-8 can directly activate downstream effector caspases including procaspase 3, 6 and 7 (Cohen, 1997). Active caspase-8 initiates downstream cleavage of caspase-3 by direct or mitochondrial-dependent mechanisms leading to apoptosis (Kuwana et al., 1998; Stennicke et al., 1998). In addition, activated caspase-3 cleave procaspase-8 (Slee et al., 1999; Woo et al., 1999), thereby amplifying the death process. It has been reported that caspase-3, an effector caspase, was marked in a few fragmented cells at 24 h following injury. Activated caspase-8 is known to propagate the apoptotic signal either by directly cleaving and activating downstream caspases or by cleaving the BH3 Bcl2-interacting protein, which leads to the release of cytochrome C from mitochondria (Oh, 2005), triggering activation of caspase-9 in a complex with dATP and Apaf-1. Activated caspase-9 then activates further downstream caspases, including caspase-8.

In SCI, some cells die by a mechanism resembling apoptosis, as is evident by caspase activation (Hara et al., 1997; Endres et al., 1998; Namura et al., 1998;
Procaspase-8 cleavage was reported by Velier et al., (1999) in mouse cortical gray matter neurons after permanent middle cerebral artery occlusion. Hence, ischemia triggers caspase 8 cleavage in spinal cord as well as within brain. As early as 1.5 hr after transient ischemia, activated caspase-8 mRNA appeared within neurons in intermediate gray matter and in medial ventral horn (Matsushita et al., 2000). Oxidative stress can lead to the activation of caspase 8 (Baumgartner et al., 2007).

**Bax**

Apoptosis can be controlled by the degradation rate of proapoptotic proteins such as Bax (Li and Dou, 2000). Bax is otherwise known as Bcl-2 associated protein X. Bax belongs to Bcl-2 family of proteins. It was the first identified pro-apoptotic member of the Bcl-2 protein family (Oltvai et al., 1993). Bax is found in the cytosol, but upon initiation of apoptotic signaling, it undergoes a conformation shift and inserts into organelle membranes, primarily the outer mitochondrial membrane (Wolter et al., 1997). Thus, it is a major player in the mitochondrion form of apoptosis (Kroemer and Reed, 2000). Mitochondria mediated cell death involve down-modulation of Bax antagonists such as Bcl-XL or Bcl-2 (Vander & Thompson, 1999) or the translocation of Bax from the cytosol to mitochondria (Khaled et al., 1999). Mitochondria dysfunction promoted by Bax translation leads to the leakage of cytochrome C from mitochondria (Costantini et al., 2000). Bax promote apoptosis by the release of downstream apoptogenic factors (Wei, 2001). Bax is activated by tumor suppressor protein p53.

Knockout studies in mice have shown that the presence of Bax is necessary for the execution of the apoptotic program (Cheng et al., 2001; Wei et al., 2001; Zong et al., 2001). Bax has been reported to be up regulated following ischemia induced retinal injury in rat (Kaneda, 1999). It is also elevated in intraocular pressure in murine glaucoma model (Ji, 2005). An increase in Bax
oligomerization leads to mitochondria mediated caspase activation (D Lee et al., 2008). Bax can be an important mediator of anticancer drug-induced cell death. Neurons that are lacking in Bax are protected against apoptosis (White et al., 1998). The decrease in Bax protein can reduce apoptosis in hemisection induced SCI (Han et al., 2012; Wang et al., 2012).

**Tumour Necrosis Factor α**

TNFα, a proinflammatory cytokine which is best known for its role in immune and vascular responses, can induce apoptosis in nonimmune tissues via the death domain of its cell surface receptor, TNF-R1. TNFα is activated during extrinsic pathway of apoptosis. It is found either as a 27-kDa membrane-bound precursor or a 17-kDa mature soluble form produced by the protease action of TNFα converting enzyme and MMP-9 (Kherif et al., 1999; Mullberg et al., 2000). 17-kDa TNFα form, which correlates with higher proteolytic cleavage is increased during active myonecrosis (Leite et al., 2010). One of the most important biological triggers of oligodendrocyte apoptosis in SCI is TNFα. In particular, it has been shown that TNFα induces apoptosis in oligodendrocytes both *in vitro* and *in vivo* (Muzio et al., 1997) by the activation of caspase-3 and caspase-8 (Hisahara et al., 1997).

There is a large amount of evidence that TNFα and IL-1β also play an important role in the induction of iNOS (inducible Nitric Oxide Synthase), which is known to play an important role in the development of SCI (Matsuyama et al., 1998). The inflammatory cytokine mRNAs were shown to be induced as early as 15 min following contusion of rat spinal cord, with increased TNFα (Pan et al., 2002; Lammertse et al., 2004). Previous studies suggests that 30-45 min post SCI, TNFα positive cells are seen over the injured spinal cord segment and from 3 to 24 h, TNFα was strongly up regulated around the contused area (Habgood et al., 2007). TNFα could potentiate glutamate mediated neuronal cell death in the rat
spinal cord (Bracken et al., 1997; Hermann et al., 2001), while TNFα antagonist
reduced the development of inflammation and tissue injury events associated with
SCI (Xu et al., 1998; Genovese et al., 2006). Etanercept (TNF α antagonist)
reduces the associated tissue damage of SCI, improves hindlimb locomotor
function, and facilitates myelin regeneration. This positive effect of etanercept on
SCI is attributable to the suppression of TNFα and caspase-8, there by inhibiting
neuronal and oligodendroglial apoptosis. (Chen et al., 2007). However, there are
conflicting reports as to the role of cell death in SCI that probably reflect the
known capacity of TNF to be both pro and anti apoptotic (Inukai et al., 2009;
Cantarella et al., 2010; Genovese et al., 2010).

**Nuclear factor kappa-light-chain-enhancer of activated B cells**

NFκB is a transcription factor that plays important roles in the immune
system (Ghosh et al., 1998; Li & Verma, 2002; Bonizzi & Karin, 2004). Known
inducers of NF-kB activity include reactive oxygen species (ROS), TNFα, IL-1β,
bacterial lipopolysaccharides, isoproterenol, cocaine and ionizing radiation. ROS
enhance the signal transduction pathways for NF-kB activation in the cytoplasm
and translocation into the nucleus. NF-kB regulates the expression of cytokines,
cyclooxygenase 2, growth factors, inhibitors of apoptosis and effector enzymes in
response to ligation of many receptors involved in immunity including T-cell
receptors, B-cell receptors and members of the Toll-like receptor/IL-1 receptor
super family.

In mammals, the NF-kB family is composed of five related transcription
factors: p50, p52, RelA (p65), c-Rel and RelB (Moynagh, 2005; Hoffmann et al.,
2006). The Rel/ NF-κB family of transcription factors are involved mainly in
stress-induced, immune and inflammatory responses. In its inactive form, NF-κB
is sequestered in the cytoplasm, bound by members of the IkB family of inhibitor
proteins, which include IkBa, IkBβ, IkBγ, and IkBe. Thus, it exists in the cytosol
as a pre-formed trimeric complex. The various stimuli that activate NF-κB cause phosphorylation of IkB, which is followed by its ubiquitination and subsequent degradation. NF-κB is trapped in the cytoplasm in stimulated cells and translocates into the nucleus in response to several stimuli, including oxidative stress. There are two signaling pathways leading to the activation of NF-κB known as the canonical pathway (or classical) and the non-canonical pathway (or alternative pathway) (Karin, 1999; Gilmore, 2006; Scheidereit, 2006; Tergaonkar, 2006).

Functional NF-κB complexes are present in essentially all cell types in the nervous system, including neurons, astrocytes, microglia and oligodendrocytes (O’Neill & Kaltschmidt, 1997). Neurons and their neighboring cells employ the NF-κB pathway for distinctive functions as well, ranging from development to the coordination of cellular responses to injury of the nervous system and to brain specific processes such as the synaptic signaling that underlies learning and memory. NF-κB also plays a role in the development and the activity of a number of tissues including the CNS (Memet, 2006). NF-κB is also an important regulator in cell fate decisions, such as programmed cell death and proliferation control and is critical in tumorigenesis (Baldwin et al., 1996). The most potent NF-κB activators are the proinflammatory cytokines IL-1 and TNFα. NF-κB is a ubiquitous transcription factor. It is activated during pathological conditions (Kaidashev, 2012).

Microglial cells can produce neurotoxic ROS and excitotoxins when activated. Cytokine mediated activation of microglia explains the ability of inhibitors of NF-κB to protect against cell damage in certain experimental paradigms that involve an inflammatory response (Qin et al., 1998). Microglial activation is associated with a marked increase in expression of cyclooxygenase-2 (Cox), an oxyradical-generating enzyme and agents that inhibit NF-κB can
suppress lipo polysaccharide induced Cox-2 expression, suggesting an important role for NF-κB in microglial activation and oxyradical production.

Activation of NF-κB in astrocytes results in increased expression of NOS and increased nitric oxide production. A potent inducer of NF-κB activation in astrocytes is bradykinin, an inflammatory mediator produced in the brain in response to ischemia and trauma (Schwaninger et al., 1999). Acting through an NF-κB mediated pathway, bradykinin induces production in astrocytes of IL-6, which stimulates production of several inflammation related cytokines. Immunohistochemical studies suggest that levels of NF-κB activity are increased in cholinergic neurons in the basal forebrains of Alzheimer’s Disease patients (Boissiere et al., 1997).

Implicated in multiple biochemical pathways affected after SCI is the transcription factor NF-κB. In traumatic SCI in the rat, both NF-κB and the iNOS are activated in microglia and neurons within and surrounding the injury site (Bethea et al., 1998). NF-κB signaling pathway operating in astrocytes is a major contributor to the pathological events occurring after SCI. Spinal cords of patients with Amyotrophic Lateral Sclerosis show increased NF-κB activation in astrocytes associated with degenerating motor neurons (Migheli et al., 1997). SCI initiates a very robust inflammatory response, both within the spinal cord and systemically. The anti apoptotic role of NF-κB in developing neurons is seen in the mechanism whereby the protein synthesis inhibitor cycloheximide prevents neuronal apoptosis. Levels of cycloheximide that cause only a small impairment of protein synthesis can prevent apoptosis by inducing Bcl-2 and the antioxidant enzyme Mn-SOD (Manganese Superoxide Dismutase) (Furukawa et al., 1997).

OXIDATIVE STRESS AND SPINAL CORD INJURY

Oxidative stress is considered as a hallmark of SCI (Jia et al., 2012). The oxidative stress by induction of ROS initiates a cascade of oxidative events that
lead to cell death due to a combination of necrosis and apoptosis (Crowe et al., 1997). Local and systemic inflammatory response, as well as neurodegenerative disease, is also associated with the production of ROS such as superoxide anion \( \text{O}_2^- \), Hydrogen peroxide \( \text{H}_2\text{O}_2 \) and peroxynitrite (Cuzzocrea et al., 2001). Oxygen free radical formation and lipid peroxidation enhance adverse mechanism of neuronal injury, such as spinal cord hypoperfusion, development of oedema, axonal conduction failure and breakdown of energy metabolism. Dusart & Schwab (1993) demonstrated that neutrophils and macrophages enter the spinal cord after SCI in an orchestrated temporal sequence. Neutrophils are able to release reactive oxygen and nitrosyl radicals as well as cytokines, chemokines and a variety of enzymes. Several studies have implicated the formation of ROS and reactive species of nitrogen in the secondary neuronal damage of SCI (Xu et al., 2001). The importance of free radicals and peroxidation in SCI is supported by the large number of experimental and clinical studies demonstrating potential neuronal efficacy of agents with anti-oxidant proprieties (La Rosa et al., 2004; Genovese et al., 2005b; 2006a,b; Scott et al., 2005). SCI initiates a sequence of events that lead to secondary neuronal cell damage. While the precise mechanisms responsible to damage in SCI remain undefined, several studies have implicated ROS in the secondary neuronal damage of SCI (Liu et al., 1997; Xu et al., 2001). ROS and peroxynitrite also cause DNA damage (Salgo et al., 1995; Szabo et al., 1997; Szabo et al., 1998). Suppression of ROS renders a protective effect for injured spinal cord (Suzuki et al., 2005). Thus, alleviating oxidative stress is an effective way of therapeutic intervention of SCI (Jia et al., 2012). The reactive oxygen intermediates produced in mitochondria, peroxisomes and the cytosol are scavenged by cellular defending systems including enzymatic SOD, GPx, glutathione reductase, catalase and nonenzymatic antioxidants (ex. glutathione, thioredoxin, lipoic acid, ubiquinol, albumin, uric acid, flavonoids, vitamins A, C and E). Antioxidants are located in cell membranes, cytosol and in the blood plasma (Maritim et al., 2003).
SUPER OXIDE DISMUTASE

SOD is an oxygen radical scavenger, which converts the superoxide anion radical present in the upper stream of reactive oxygen metabolism cascade and afford protection from cell damage. It is found in almost all organisms living in the presence of oxygen, including some anaerobic bacteria, supporting the notion that superoxide is a key and general component of oxidative stress. In aerobic cells, free radicals are constantly produced mostly as ROS. SOD catalyses the dismutation of the O$_2^-$ into H$_2$O$_2$ (Michiels et al., 1994). Imbalance between pro oxidant and anti- oxidant defenses in favour of pro oxidants results in oxidative stress. This results in damage to lipids, proteins and nucleic acids. Alone or in combination with primary factors, free radicals are involved in the cause of hundreds of diseases. There are two types of SOD: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. Cu/Zn SOD protects the cell’s cytoplasm and Mn SOD protects their mitochondria from free radical damage. Highly reactive oxygen-containing species form upon CNS injury and cause oxidative damage to important cellular components, thereby destroying cells. Removal of superoxide may be a realistic treatment strategy for reducing injury caused by free radicals (Liu et al., 1998).

Free radical mediated mechanisms of cellular damage have been implicated in the early stages of SCI. Superoxide radicals contribute to the pathogenesis of SCI (Taoka et al., 1995). Mn SOD is a potent scavenger of superoxide radicals and likely serves an important cytoprotective role in preventing cellular damage after SCI. SOD has been reported to promote functional recovery in ischemic SCI. SOD treatment, targeted to the early reperfusion period, reduced both motor dysfunction and incidence of spinal infarcts at 7 days after ischemia (Cuevas et al., 1990).
GLUTATHIONE PEROXIDASE

GPx is a selenoprotein enzyme found in cytoplasmic and mitochondrial fractions of cells. The antioxidant enzyme catalyzes the reduction of H$_2$O$_2$ and hydroperoxides formed from fatty acids, thereby effectively removing toxic peroxides from living cells (Michiels et al., 1994). It plays the important role of protecting cells from potential damage by free radicals, formed by peroxide decomposition (Mannervik et al., 1985; Ursini et al., 1985). The activity of GPx is coupled to glutathione reductase, which maintains reduced glutathione levels (Bompart et al., 1990). Enzyme activity can be decreased by negative feedback from excess substrate or from damage by oxidative modification (Tabatabaie et al., 1994). Decreased GPx activity has been reported in tissues where oxidative stress occurs. Therefore GPx activity is necessary for reducing the oxidative stress.

Tissue damage is induced by ROS in the primary and secondary process of SCI (Ikeda & Long 1990). GPx is involved in scavenging of free radicals. Absence of Gpx leads to neuronal apoptosis (Crack et al., 2003). Neuronal apoptosis is attributed partly to diminished activation of Akt (Taylor et al., 2006). Thus GPx indirectly affects neuronal cell survival. GPx protects cortical cells from oxidative injury (Ran et al., 2006). Stimulation of GSH dependent antioxidative processes lead to reduced oxidative damage and greater locomotor function during the sub acute and chronic phases of injury (Richard et al., 2006). Induction of oxidative stress results in decreased GPx, so decreasing oxidative stress by antioxidant agents play a key role in attenuating SCI (Ayromlou et al., 2011).

NEURONAL SURVIVAL FACTORS IN SPINAL CORD INJURY

Neurogenesis is the life-long natural production and integration of new nerve cells in the brain. Neurons and glia are in close contact in the mature nervous systems of all animals. It has been suggested that all cells are
programmed to die unless they receive trophic support and that cell survival largely depends upon interactions between cells (Raff et al., 1993). Both neurons and glia are overproduced in the normal nervous system and there is evidence indicating that one consequence of axon-glial contact is survival regulation in both cell types in the mature nervous system (Raff et al., 1993). This ensures that axons are correctly myelinated, enabling normal neuronal function.

Neurotrophic factors (NTF) play an important role in the maintenance of structural integrity of the mature brain as well as the development of the CNS. Previous cell culture experiments have shown that several groups of growth factors exert trophic actions on CNS neurons by promoting survival and their morphological and biochemical differentiation. Among them are included the IGFs (Knusel et al., 1990), GDNF (Lin et al., 1993) and the neurotrophin family, BDNF. Neurotrophins regulate development, maintenance, and function of vertebrate nervous systems (Eide et al., 1993; Korsching, 1993; Lewin & Barde, 1996; Segal & Greenberg, 1996; Reichardt & Farinas, 1997; McAllister et al., 1999; Sofroniew et al., 2001). All neurotrophins have six conserved cysteine residues and share a 55% sequence identity at the amino acid level. Neurotrophins activate two different classes of receptors, the Trk family of receptor tyrosine kinases and p75NTR, a member of the TNF receptor superfamily. Through these, neurotrophins activate many signaling pathways, including those mediated by ras and members of the cdc-42/ras/rho G protein families, and the MAP kinase, PI-3 kinase, and Jun kinase cascades. During development, neurotrophins function as survival factors to ensure a match between the number of surviving neurons and the requirement for appropriate target innervation. They also regulate cell fate decisions, axon growth, dendrite pruning, the patterning of innervation and the expression of proteins crucial for normal neuronal function, such as neurotransmitters and ion channels. These proteins also regulate many aspects of neural function. In the mature nervous system, they control synaptic function and
synaptic plasticity, while continuing to modulate neuronal survival. Growth factors such as Nerve Growth Factor (NGF), BDNF, neurotrophin-3, ciliary neurotrophic factor, and GDNF all have been used to study their beneficial effects in spinal cord–injured animals (Lu & Tuszynski, 2008). IGF is activated by Akt and promotes neuronal survival. Cyclin D1 is a marker of proliferation and is in turn activated by IGF.

**BRAIN DERIVED NEUROTROPHIC FACTOR**

BDNF is a member of the neurotrophin family of growth factors that includes NGF, NT-3, and NT-4. It is a 13.6 kDa (or 27.2 kDa dimer) member of the neurotrophin family. The active form of recombinant human BDNF (27 kDa) is a dimer formed by two identical 119 amino acid subunits held together by strong hydrophobic interactions plays a key role in neuronal and axonal survival. BDNF has identical amino acid sequence in human, mouse and pig with full cross-reactivities. BDNF is regulated by another neurotrophin, NGF.

BDNF, a survival-promoting molecule, plays an important role in the growth, development, maintenance and function of several neuronal systems. Several populations of sensory neurons have been shown to synthesize BDNF (Brady et al., 1999; Mannion et al., 1999). Although some evidence has been presented suggesting that BDNF act in an autocrine or paracrine fashion to support DRG sensory neurons (Acheson et al., 1995; Robinson et al., 1996), in other instances it may be transported anterogradely and act trans-synaptically on targets of the central afferents of these neurons within the brain (von Bartheld et al., 1996; Altar et al., 1997; Fawcett et al., 1998; Brady et al., 1999).

BDNF is important in mature animals for regulating the mechanosensitivity of slowly adapting mechanoreceptors, myelinated fibers required for fine tactile discrimination (Carroll et al., 1998). It is constitutively expressed by adult sensory neurons (Ernfors et al., 1990; Apfel et al., 1996; Cho
et al., 1997; Michael et al., 1997). BDNF has been shown to enhance the survival and differentiation of several classes of neurons in vitro, including neural crest and placode derived sensory neurons, dopaminergic neurons in the substantia nigra, basal forebrain cholinergic neurons, hippocampal neurons, and retinal ganglial cells. It promotes the growth of GABAergic neurons of the striatum and ganglion cells of the retina and neural crest. It also protects motor neurons, cortical neurons and the hippocampus from various forms of toxic damage. It has a functional role in autoimmune demyelination by mediating axonal protection (Ralf et al., 2010). It is involved in normal maturation of neuronal pathways and regulates neuronal plasticity. The versatility of BDNF is emphasized by its contribution to a range of adaptive neuronal responses including long-term potentiation, long-term depression, certain forms of short-term synaptic plasticity, as well as homeostatic regulation of intrinsic neuronal excitability (Egan et al., 2003). Changes in the levels and activities of BDNF have been described in a number of neurodegenerative disorders, including Huntington’s disease, Alzheimer’s disease and Parkinson’s disease.

In the lumbar DRG of the rat, the distribution of BDNF protein is not uniform but is localized to a restricted number of primary sensory neurons. The type of neurons that synthesize BDNF has been elucidated (Michael et al., 1997). It has emerged that BDNF play an important neuro modulatory role in the dorsal horn of the spinal cord. It is markedly up regulated in inflammatory conditions in a NGF dependent fashion. Postsynaptic cells in this region express receptors for BDNF. The increase in BDNF mRNA reduced motor functional deficits in spinal cord transaction rats (Gao et al., 2012; Jin et al., 2012). BDNF results in enhanced connectivity of the peripheral motor bridge in a rodent model of SCI (Martin et al., 2012). BDNF has a neuroprotective effect in SCI (Uchida et al., 2012).
GLIAL DERIVED NEUROTROPHIC FACTOR

GDNF, a member of the TGFβ superfamily (Lin et al., 1993), is a NTF that promotes the survival of various neuronal populations in both the central and peripheral nervous systems during their development. It is the most potent motor NTF among NTFs found so far. GDNF binds to glial cell-derived neurotrophic factor receptor (GFR) α-1, a membrane-bound protein belonging to the GFRα family (Jing et al., 1996; Treanor et al., 1996). Cells known to express GDNF include sertoli cells, type1 astrocytes, schwann cells, neurons, pinealocytes and skeletal muscle cells. It maintains dopaminergic, noradrenergic and motor neurons of the CNS (Lin et al., 1993; Henderson et al., 1994; Arenas et al., 1995; Oppenheim et al., 1995; Tomac et al., 1995; Yan et al., 1995) as well as various sub populations of the peripheral sensory and sympathetic neurons (Henderson et al., 1994; Buj-Bello et al., 1995; Ebendal et al., 1995; Trupp et al., 1995).

The most prominent feature of GDNF is its ability to support the survival of dopaminergic and motor neurons. In addition, exogenously applied GDNF has been shown to rescue damaged facial motor neurons in vivo. GDNF play an important role in corneal regeneration and wound healing (You et al., 2001). GDNF mRNA expression in schwann cells in sciatic nerves and in DRGs rises dramatically, a finding that implicates GDNF in peripheral nerve regeneration (Trupp et al., 1995; Hammarberg et al., 1996; Hoke et al., 2000, 2002). GDNF mRNA expression is also increased in an experimental model of motor neuropathy in rats (Saita et al., 1997), in various human neuropathies (Yamamoto et al., 1997) and in traumatized human nerves (Ba’r et al., 1998). Members of the GDNF family of ligands do play essential early roles in development of one sympathetic ganglion, the superior cervical ganglion. There is a hope that GDNF might become useful in the treatment of neurodegenerative diseases and nerve injuries.
GDNF is one of the most powerful survival factors for spinal motor neurons (Chu et al., 2012). The primary sensory neurons that respond to noxious stimulation and project to the spinal cord are known to fall into two distinct groups: one sensitive to NGF and the other sensitive to GDNF (Snider & McMahon, 1998). GDNF has been shown to protect cranial and spinal motoneurons. GDNF promote spinal repair and functional recovery (Li et al., 2004). There is evidence that exogenous GDNF could reduce the hyperplasia of glial cells and enhance the regeneration of injured corticospinal tract and restoring of the cytoskeleton in the injured neurons (Lu et al., 2002). GDNF induced the growth of motor and sensory axons and remyelination in laboratory rats with partial and complete spinal cord transections (Blesch & Tuszynski, 2001; Zhou et al., 2003).

**Insulin like Growth Factor-1**

IGFs are peptide hormones secreted from many different cells. IGF has been shown to play roles in the promotion of cell proliferation and the inhibition of cell death (apoptosis). There are two principle IGFs referred to as IGF-I and IGF-II. IGF-I is developmentally regulated such that peak levels coincide with neuronal proliferation and neurite outgrowth (D’Ercole et al., 1996; Ye et al., 1997; Dentremont et al., 1999), whereas IGF-II is mainly expressed in cells of mesenchymal and neural crest origin (D’Ercole et al., 1996). IGF-1 promotes cell survival (Segal & Greenberg, 1996; Stewart & Rotwein, 1996; Ataliotis & Mercola, 1997). The signaling of the IGF-1 receptor is activated through Akt in oligodendrocytes. IGF-1 signaling through Akt enhances oligodendrocyte progenitor cell survival after glutamate exposure (Ness & Wood, 2002; Ness et al., 2002), growth factor deprivation (Cui et al., 2005). IGF-I has an involvement in regulating neural development including neurogenesis, myelination, synaptogenesis and dendritic branching and neuroprotection after neuronal damage. It is physiologically important for the development of granule neurons.
(Bondy et al., 1991; Gao et al., 1991; Calissano et al., 1993; Ye et al., 1996). It promotes the *in vitro* survival and neurite outgrowth of various sensory, sympathetic, cortical and motor neurons (Aizenman & de Vellis, 1987; Caroni & Grandes, 1990, Svrizic & Schubert, 1990; Bozyczko-Coyne et al., 1993; Neff et al., 1993).

Estrogen receptors and IGF-I receptors interact in the promotion of neuronal survival and neuroprotection (Garcia-Segura et al., 2000). IGF influences neuronal differentiation (Levi-Montalcini et al., 1987; Barde, 1989; Ferrari et al., 1989; Maisonpierre et al., 1990). IGF-I mRNA is widely expressed in the vertebrate brain (Bartlett, 1991; Bondy, 1991). IGF-I and cAMP can protect cerebellar granule neurons from apoptosis in low K+. It is known that IGF-I is synthesized and secreted by cerebellar Purkinje cells (Andersson et al., 1988; Bondy, 1991). Further, the IGF-I receptor is present in granule neurons (Lesniak et al., 1988; Marks et al., 1990; Bondy, 1991). IGF-I therefore serve as a survival factor for these neurons in vivo.

IGF-I has been shown to be a potent NTF that promotes the growth of projection neurons, dendritic arborization and synaptogenesis. Patients with SCI have been reported to have lower plasma IGF-I. IGF-1 protects motor neuron cells from ischemic SCI associated with differential regulation of Bcl-xL and Bax protein (Nakao et al., 2001). Subcutaneous administration of IGF-I resulted in better locomotor recovery following SCI (Koopmans et al., 2006). IGF-I attenuates caspase-9 cleavage, increases Bcl2, thus inhibiting apoptosis after SCI (Hung et al., 2007). BDNF and IGF pre treatment is neuroprotective in SCI and that these NTF have the capacity to down regulate NOS expression following trauma to the spinal cord (Sharma et al., 1998).
Akt

The serine–threonine kinase Akt (also known as protein kinase B) is a central convergence node in a broadly influential signaling network. Akt activation serves as a master switch of these cellular signaling pathways, generating a multitude of intracellular responses through a plethora of downstream targets and interacting partners.

Akt is expressed as three isoforms — AKT1/ PKBα, AKT2/ PKBβ and AKT3/ PKBγ, respectively (Vivanco & Sawyers, 2002). An amino terminal pleckstrin homology (PH) domain, a central serine–threonine catalytic domain, and a small carboxy-terminal regulatory domain characterize all the three isoforms. The PH domain binds to PIP2 and PIP3, products of PI3K. This binding causes Akt to locate to the plasma membrane, where it becomes phosphorylated by phosphoinositide-dependent kinase 1 on Thr308 in the activation loop of the catalytic domain. This phosphorylation leads to activation. Full activation requires phosphorylation at a second site (Ser473). Current evidence leads to the mTOR–rictor complex as the primary kinase for the second phosphorylation event, although other kinases like integrin linked kinase (Ilk) (Persad et al., 2000), phosphoinositide-dependent kinase 1 (Balendran et al., 1999), DNA-dependent protein kinase (DNA-PK) (Feng et al., 2004), ataxia telangiectasia mutated (ATM) have also been identified (Dong & Liu, 2005).

Akt is a general mediator of growth factor induced survival and has been shown to suppress the apoptotic death of a number of cell types induced by a variety of stimuli, including growth factor withdrawal, cell-cycle discordance, loss of cell adhesion and DNA damage (Ahmed et al., 1997; Dudek et al., 1997; Kauffmann-Zeh et al., 1997; Kennedy et al., 1997; Khwaja et al., 1997; Kulik et al., 1997). Thus cellular processes regulated by AKT include cell proliferation and survival, cell size and response to nutrient availability, intermediary metabolism,
angiogenesis and tissue invasion. Growth factor receptor activation leads to the sequential activation of PI3K (Phosphatidylinositol-3-OH kinase)/Akt, which then, promotes cell factors such as IGF-1. Growth factor activation of the PI3K/Akt signaling pathway also culminates in the phosphorylation of the Bcl-2 family member Bad and caspase 9, thereby suppressing apoptosis and promoting cell survival (Sandeep et al., 1997; Nakanishi et al., 2005). Alternatively, inhibition of Akt promotes phosphorylation of the proapoptotic Bad, which favours the apoptotic process (Cardone et al., 1998). It also affects the transcriptional response to apoptotic stimuli by acting on Forkhead factors and also influences the activity of the p53 family (Brunet et al., 1999; Ogawara et al., 2002; Karen et al., 2008; Zhaohui, 2009). Akt phosphorylation has been considered a critical factor in the aggressiveness of cancer. Quercetin induced inactivation of Akt contributing to the promotion of apoptosis.

Akt is increasingly recognized as an important regulator of signal transduction pathways and play important roles in functional recovery after nervous system injury. A significant activation of the Akt/mTOR/p70S6K signaling pathway in the injured spinal cord produces beneficial effects on SCI induced motor function defects and repair potential (Hu et al., 2010). Phospho-Akt and phospho-Bad were colocalized in motor neurons that survived SCI and inhibition of PI3K reduced expression of phospho-Akt and phospho-Bad (Yu et al., 2005). Oxidative stress plays a role in modulating Akt/Bad signaling and subsequent motor neuron survival after SCI (Yu et al., 2005). PI3 kinase/Akt pathway mediates oligodendrocyte progenitor cell survival and proliferation and survival of mature oligodendrocytes induced by numerous compounds (Canoll et al., 1999; Ebner et al., 2000; Flores et al., 2000; Ness and Wood, 2002; Baron et al., 2003; Jaillard et al., 2005; Cui et al., 2006; Pang et al., 2007).
CYCLINS

Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. Cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. Together with its binding partners cyclin dependent kinase 4 and 6 (CDK4 and CDK6), cyclin D1 forms active complexes that promote cell cycle progression by phosphorylating and inactivating the retinoblastoma protein (RB) (Kato et al., 1993; Weinberg, 1995; Lundberg & Weinberg, 1998). Cyclin D1 encodes the regulatory subunit of a holoenzyme that phosphorylates and inactivates the retinoblastoma protein. Amplification or overexpression of cyclin D1 plays pivotal roles in the development of a subset of human cancers including parathyroid adenoma, breast cancer, colon cancer, lymphoma, melanoma and prostate cancer. The cyclin D1 proto-oncogene is an important regulator of G1 to S phase progression in many different cell types.

Cyclin D1 associates with and regulates activity of transcription factors, coactivators and corepressors that govern histone acetylation and chromatin remodeling proteins. The recent findings that cyclin D1 regulates cellular metabolism, fat cell differentiation and cellular migration. Cyclin D1 rises rapidly after induction of proliferation (Tang et al., 2001; Huang et al., 2002). Frederick et al., (2007). Studies have demonstrated that cyclin D1 also functions as transcriptional modulator by regulating the activity of several transcription factors and histone deacetylase (HDAC3) (Coqueret, 2002). This activity is independent of CDK4 activity. The cyclin D1 protein has been shown to be unstable with a short half-life (~24 min) (Diehl et al., 1997; Diehl et al., 1998) and is degraded mainly via the 26S proteasome in a ubiquitin-dependent manner (Diehl et al., 1997). Early studies suggested that the Skp2 F-box protein might be involved in cyclin D1 degradation (Yu et al., 1998). Recently, two further F-box proteins were
identified in separate studies as playing major roles in targeting the cyclin for degradation (Lin et al., 2006; Okabe et al., 2006).

Cyclin D1 plays a central role in the regulation of proliferation, linking the extracellular signaling environment to cell cycle progression (Sherr, 1995). The expression level of cyclin D1 is highly responsive to the action of proliferative signals including growth factor receptors, Ras and their downstream effectors. Regulation in expression level involves a variety of mechanisms including production, stability and utilization of cyclin D1 mRNA; as well as protein stability, localization and association. Its expression increases upon stimulation of quiescent cells to enter the cell cycle, while it has been proposed to shuttle in and out of the nucleus through the cell cycle of actively cycling cells (Aktas & Cooper, 1997). The evidence indicates that cyclin D1 levels not only regulate the initiation of cell cycle progression in quiescent cells, but that they play a critical role in the decision of a cell to continue proliferating. This decision is made during G2 phase when proliferative signaling induces an increase in cyclin D1 levels. The apparently automatic decline in cyclin D1 levels during the preceding S phase allows efficient DNA synthesis and ensures that proliferative conditions are conducive for continued growth at the time of commitment for continuing proliferation during G2 phase.

Cyclin D2 is a member of the family of D-type cyclins that is implicated in cell cycle regulation, differentiation and oncogenic transformation. cyclinD2 provides neuro protection (Cernak et al., 2005; Di Giovanni et al., 2005; Tian et al., 2006). Forced maintenance of cyclin D2 favours proliferation at the expense of neuronal differentiation. Mice expressing only cyclin D3 lack normal cerebella while mice expressing only cyclin D2 present neurological abnormalities (Ciernyech et al., 2002). Previous studies have also revealed the expression of cyclin D1 and D2 in post-mitotic cells of the developing nervous system (Tamaru et al., 1993; De Falco et al., 2004; Schmetsdorf et al., 2005, 2006). High levels of
cyclin D2 mRNA were localized in S-phase cells. However, this would imply that in these neural progenitors Cyclin D2 has a function only in the daughter cells during the next cell cycle (Salles et al., 2007). Cyclin D2 has also been linked to cancer, as abnormal expression of this gene also correlates with tumor development (Hanna et al., 1993; Siciniski et al., 1996). Cyclin D2 degradation has a role in many diseased conditions (Bill et al., 2012). Overexpression of cyclin D2 protein efficiently inhibited cell cycle progression and DNA synthesis (Muthupalaniappan et al., 1998).

Up-regulation of cell cycle proteins occurs after CNS trauma (Di Giovanni et al., 2003), and is associated with apoptotic cell death of post-mitotic cells such as neurons and proliferation of astrocytes and microglia (Becker & Bonni, 2004). Cell cycle proteins are normally down-regulated in post-mitotic neurons (Okano et al., 1993) and re-entry into the cell cycle can cause apoptosis in such cells (Nguyen et al., 2002; Becker & Bonni, 2004). Neurons are typically described as terminally differentiated, permanently held at the G0 phase of the cell cycle, but findings have demonstrated that post-mitotic neurons attempt to re-enter the cell cycle in pathological circumstances (Timsit et al., 1999; Osuga et al., 2000 Malik et al., 2008). Up-regulation of cell cycle proteins is correlated with neuronal apoptosis after experimental SCI (Di Giovanni et al., 2003) and brain injury (Natale et al., 2003). Induction of cyclin D1 and Cdk4 may be implicated in programmed cell death change after transient spinal cord ischemia in rabbits (Sakurai et al., 2000). Administration of cell cycle inhibitors such as flavopiridol, roscovitine or olomoucine can provide neuroprotection in various in vitro models, such as etoposide, kainic acid or colchicine induced injury (Jorda et al., 2003; Cernak et al., 2005; Di Giovanni et al., 2005). Flavopiridol also blocks astrocyte and microglial proliferation in vitro (Cernak et al., 2005; Di Giovanni et al., 2005; Wu et al., 2012).
CELL THERAPY IN SCI

Cell therapy is a highly hopeful method in clinical applications, raising so much optimism for the treatment of injured tissues with no self regeneration potential such as central and peripheral nervous system. A promising strategy in SCI repair being developed is stem cell or progenitor cell based therapy (Hwang et al., 2011). It is hoped that these undifferentiated cells will provide an inexhaustible source of neurons and glia for therapies aimed at cell replacement or neuroprotection in disorders affecting the CNS. The transplantation of stem cells could promote functional recovery by reconstituting damaged nerve tracts, remyelinating axons and increasing plasticity or axon regeneration (Moraleda et al., 2006). Indeed, several studies have reported partial functional improvements and fiber regeneration following spinal implantation of different types of cells (Garbuzova-Davis et al., 2009). Locomotor improvements have been demonstrated in SCI mice and primates (Watanabe et al., 2004; Cummings et al., 2005; Iwanami et al., 2005). However, no single therapeutic methodology has been proven effective enough for significant functional recovery because of difficulties such as post transplantation cellular lineage restriction (Reier, 2004), variable host responses to the engraftment (Ourednik & Ourednik, 2004 a,b), and unfavorable hostile environment for the implanted cells.

BONE MARROW CELLS

Transplantation of BMCs into the injured spinal cord has been found to improve neurologic functions in experimental animal studies. Cell transplantation technology has demonstrated that BMCs differentiate into mature neurons or glial cells under specific experimental conditions and that the transplantation of BMCs can promote functional improvements after SCI (Jung et al., 2011). The hematopoietic or mesenchymal stem cells has a neuroprotective effect and increased neurite outgrowth (Ankeny et al., 2004; Chen et al., 2005). It was seen
that the mesenchymal stem cells are predisposed to differentiate into neuronal cells once the proper conditions are given. When transplanted into the CNS, they can develop into a variety of functional neural cell types, making them a potent resource for cell-based therapy. However, to date neural stem/progenitor cells transplantation has exhibited only limited success in the treatment of chronic SCI.

BMCs can support the natural regeneration processes of the body by stimulating the repair of damaged tissues. BMCs differentiate into mature neurons or glial cells under specific experimental conditions (Sanchez et al., 2000; Ha et al., 2001; Munoz et al., 2003). Transplanted BMCs improve neurological deficits in the CNS injury models by generating neural cells or myelin producing cells (Chopp et al., 2000; Akiyama et al., 2002). It has been reported that the bone marrow derived cells also have the potential to develop into neural lineages, such as neurons and astrocytes, both in vivo (Kopen et al., 1999) and in vitro (Yamada et al., 2001). BMCs which are adherent in the culture of bone marrow aspirates, have already been used for the treatment of the injured spinal cord (Chopp et al., 2000) and brain. Studies suggest that transplantation of bone marrow stem cells into spinal cord lesions enhances axonal regeneration and promotes functional recovery in animal studies (Wright et al., 2010). Thus modest improvements in neurological function have been reported following BMC administration in acute CNS injury (Mahmood et al., 2004; Himes et al., 2006).