2.1 Peptide-based pharmaceutical industry

Peptide-based pharmaceutical industry is booming rapidly due to higher successful therapeutic targets followed by improved delivery methodologies. Furthermore, more than 100 peptide drug candidates are available in the clinic and a large number of preclinical development projects are ongoing worldwide. Thus, there is a growing need for a generally applicable peptide synthesis protocol, which could be used to prepare peptides with high purity and at low cost. The solid-phase peptide synthesis (SPPS) method has been considered the gold standard for decades. While, in solution phase peptide coupling reactions can be carried out under homogenous conditions using lesser amounts of amino acids and coupling reagents compared to SPPS. Therefore, peptide synthesis methods in solution phase offer economic and qualitative advantages in process development for large-scale synthesis. However, conventional solution-phase peptide synthesis has several drawbacks including time-consumption for process development and difficulties in synthesizing peptides with hydrophilic, hydrophobic, and long chains (Nilsson, 2000).

Chemical conjugated brain specific drug delivery system represents a rational drug design approach that is exploited to delivery of the drug to the target site of action i.e. brain as well as reduces the toxicity and increase the treatment efficacy (Rapoport, 1996; Drion, 1996, 1997). As it is know that drug delivery to brain is quite difficult due to the presence of the blood brain barrier (BBB), which is formed by tight junction within the capillary endothelium of the vertebrate brain (Brightman et al., 1970). Because of the presence of BBB physicochemical properties of drugs, i.e. lipophilicity and molecular weight, determine extent to which drugs can across the BBB (Reynolds, 1998).

A key pathological feature of PD is the presence of abnormal cytoplasmic inclusions called Lewy bodies. Lewy bodies contain various forms of protein, including α-synuclein, ubiquitin-protein conjugates, phosphorylated proteins, and oxidatively damaged proteins (McNaught and Olanow, 2006). They are found not only in the SN, but also in the cortex, amygdala, locus coeruleus, vagal nucleus, and the peripheral autonomic nervous system (Braak et al., 2003; Wakabayashi and Takahashi, 1997). The role of Lewy bodies in the pathogenesis of PD is unknown. Formation of Lewy bodies may be associated with a compromised ubiquitin-
proteasome system (Betarbet et al., 2005). Inhibition of proteasomes has been shown to produce Lewy bodies in rats (McNaught et al., 2004; Sherman and Goldberg, 2001).

![Diagram of Major dopaminergic pathways in the human brain](image)

**Figure 2.1** Major dopaminergic pathways in the human brain (McNaught et al., 2004).

### 2.2 Oxidative stress: focus on PD

Oxidative stress refers to an imbalance in the redox state of a cell that is caused by either the overproduction or reduced elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The primary forms of ROS are superoxide (·O₂), hydrogen peroxide (H₂O₂), and hydroxyl radical (·OH), and the primary forms of RNS are nitric oxide (·NO), peroxynitrite (ONOO⁻), nitrogen dioxide (·NO₂), and nitrogen peroxide (N₂O₃). Because of their highly reactive nature, excess levels of ROS and RNS cause oxidative damage to key cellular components, such as DNA, RNA, lipids, and proteins, there by resulting in toxicity. Under normal conditions, mitochondria are the major cellular source of ROS producing ·O₂ as a byproduct of the respiratory chain, particularly in complexes I and III (Liu et al., 2002). The second-most abundant source of ·O₂ is NADPH oxidase, which is mainly
expressed in macrophages and microglia (Block, 2008). The physiological function of 
\(-\text{O}_2\) is to kill phagocyted microorganisms, but its overproduction leads to the 
toxicity of brain cells in several disorders such as Alzheimer’s disease (AD) and PD (Block et al., 2007). Due to the potential toxicity of ROS and RNS, cells have 
developed defense mechanisms to counteract their oxidative potential and balance the 
cellular redox state. For example, superoxide dismutase (SOD), catalase (CAT), 
glutathione peroxidase (GPx), and reduced glutathione (GSH) are the primary 
endogenous antioxidants that convert reactive free radicals into less-reactive 
molecules (Surendran and Rajasankar, 2010).

For the past several decades, oxidative stress has been considered to be one of 
the most important pathogenic factors in PD. The finding that neuromelanin is a 
byproduct of the auto-oxidation of catecholamines led researchers to theorize that 
dopamine metabolism might be responsible for the sensitivity of dopaminergic 
neurons to oxidative stress (Graham, 1978). Postmortem PD brains exhibit several 
signs of elevated ROS-induced damage within the SN, including lipid peroxidation, 
DNA damage, and protein oxidation (Surendran and Rajasankar, 2010). Moreover, 
the SN of PD patients displays a reduced antioxidative capacity, with a low content of 
GPx and GSH (Martin and Teismann, 2009). This brain region also exhibits decreased 
activity of mitochondrial complex I, which suggests that an increase in ROS may be 
caused by dysregulation of the respiratory chain (Schapira et al., 1990). Interestingly, 
most of the toxins that are used to model PD, such as 6-OHDA, MPTP, paraquat, and 
rotenone, have been linked to oxidative stress. Based on the prevailing view, all of 
these toxins lead to increased ROS levels by inhibiting mitochondrial complex I 
(Abdulwahid and Ahmad, 2010). However, researchers have recently claimed that 
MPTP, paraquat, and rotenone can also cause toxicity that is independent of complex 
I inhibition (Choi et al., 2008) and that rotenone may act primarily through the 
activation of NADPH oxidase in microglia (Gao et al., 2003).

### 2.3 Mitochondrial targeted antioxidants/peptides

Mitochondrial-targeted antioxidants including mitochondrial CoQ10 (MitoQ) 
and SzetoSchiller peptide (SS-31) play an important role in modulating ROS induced 
mitochondrial permeability transition and cell death, and were found to be protective 
in several in vitro and in vivo models of ischemia, reperfusion injury and 
neurodegeneration (Armstrong, 2007). MitoQ is a derivative of CoQ10 conjugated to
triphenylalkylphosphonium ions, which promotes uptake into mitochondria, where it can combat ROS originating in mitochondria (Tauskela, 2007). MitoQ reduces ROS formation and preserves mitochondrial function after glutathione depletion, even in the cells lacking mitochondrial DNA (Lu C et al., 2007). Murphy and colleagues have developed MitoQ, which is currently in a phase II clinical trial for PD in New Zealand.

Similarly, a novel peptide antioxidant (SS-31) targeted to the inner mitochondrial membrane prevents apoptosis, necrosis, oxidative stress and inhibition of the mitochondrial electron transport chain (Szego, 2007). SS-31 protects neuronal cells against tert-butyl-hydroperoxide (tBHP) induced mitochondrial depolarization and apoptotic cell death by reducing intracellular ROS, decreasing markers of apoptotic cell death and caspase activity (Zhao et al., 2005). It decreases mitochondrial ROS production and inhibits the MPT and mitochondrial swelling. SS-31 also prevents Ca induced cytochrome c release and inhibits 3-NP-induced activation of the MPT pore and mitochondrial depolarization in isolated mitochondria (Zhao et al., 2004). It is also effective against myocardial ischemia-reperfusion injury in both ex vivo and in vivo models (Cho et al., 2007). Recently, we investigated the therapeutic effects of SS-31 in neuronal cells, stably transfected with either wildtype or mutant Cu/Zn superoxide dismutase (SOD1), and in the G93A mouse model of ALS. We found SS-31 mediated significant neuroprotection against cell death induced by hydrogen peroxide in SOD1 neuronal cells (Petri et al., 2006). Further, daily intraperitoneal injections of SS-31 (5 mg/kg), starting at 30 days of age, led to a significant improvement in survival and motor performance in the G93A transgenic mouse model of ALS. SS-31 treated G93A ALS mice showed decreased cell loss and a decrease in immunostaining for markers of oxidative stress in the lumbar spinal cord, as compared with the vehicle-treated mice. Both SS-31 and SS-20 also produce complete protection against MPTP neurotoxicity.

2.4 Therapeutic approaches for PD treatment

The pharmacological standard for the treatment of PD remains a replacement of dopamine. This is typically accomplished with the precursor of dopamine, 1,3,4dihydroxyphenylalanine (L-DOPA). The clinical effects of L-DOPA on akinesia in Parkinsonism was first presented in 1967 by Birkmayer and Hornykiewicz.
is typically administered in a combination with carbi-dopa, an inhibitor of DOPA decarboxylase which reduces the peripheral conversion of L-DOPA to dopamine thus allowing for considerable amount of the drug to cross the blood brain barrier and undergo decarboxylation to DA in the brain. Although the initial therapeutic effects of L-DOPA are excellent, patients develop drug-related side effects over the course of the disease, which include motor fluctuations (the so-called "wearing-off" and "on–off" phenomena) and dyskinesias (Lang and Lozano, 1998). Other medications including anticholinergic agents, inhibitors of catechol-Omethyltransferase (COMT) or monoamine oxidase-B (MAO-B) provide only mild-to-moderate benefit (Hristova and Koller, 2000; Olanow and Stocchi, 2004; Marjama and Koller, 2001). Eventually, L-DOPA or dopamine agonists are required for management of progressive disability. However, dopamine agonists usually take longer than L-DOPA to reach effective doses, and also require co-administration of L-DOPA for supervening disability after varying periods of time. At the later stages of the disease patients developing resistance or severe side effects to pharmacological therapy may benefit from neurosurgical procedures such as pallidotomy or deep brain stimulation of the subthalamic nucleus (STN) (Esselink et al., 2004). Nevertheless, none of the currently available treatments has been proven progression of PD.

Levodopa is the most efficacious treatment in the management of Parkinson's disease. Unfortunately, chronic use of traditional levodopa/dopa decarboxylase inhibitor formulations is associated with the development of complications, such as wearing-off and dyskinesia. In an attempt to avoid these complications, some physicians delay the introduction of levodopa or employ levodopa-sparing strategies; however, these strategies are frequently suboptimal for patients. As most patients required the superior efficacy of levodopa during the course of their disease, an appreciation of the changing response to levodopa over time and an understanding of the pharmacokinetic principles underlying the development of complications such as wearing-off is essential in the long-term management of the patient (Stocchi and Fabrizio, 2006).
2.5 Neuroprotective gene therapy: achievements and perspectives

Different strategies have been employed to inhibit neurodegenerative processes: early studies aimed at blocking the executioners of apoptotic cell death, cysteine proteases of the caspase family; however, no sustained neuroprotection could be achieved (Rideout and Stefanis, 2001). Most pro-apoptotic signals converge on breakdown of mitochondrial membrane potential, followed by release of pro-apoptotic factors and subsequent caspase activation (Chang et al., 2002). Thus, several studies focused on the maintenance of mitochondrial integrity by overexpression of anti-apoptotic members of the bcl-2 family of proteins (Malik et al., 2005; Wong et al., 2005). This strategy proved to be significantly more efficient than caspase inhibition, although in long-term studies substantial neuronal cell loss was still observed (Malik et al., 2005). Neurotrophic factors in several paradigms could only shortly postpone neuronal degeneration (Van et al., 2003). Glial cell line-derived neurotrophic factor (GDNF) appears to be an exception and remains a promising candidate in the treatment of Parkinson’s disease.

2.5.1 Glial cell line-derived neurotrophic factor (GDNF)

GDNF was originally identified as a trophic factor promoting the survival of embryonic midbrain dopaminergic neurons (Lin et al., 1993). Subsequently, it was found to be a potent trophic factor for noradrenergic neurons of the CNS (Arenas et al., 1995) as well as for the moto- and sensory neurons of the PNS (Ramer et al., 2000). GDNF was purified from a glioma cell line supernatant and then found to be
expressed in several glial cell types of the NS (Strelau and Unsicker, 1999). In toxicity animal models of PD, GDNF was proven to rescue DA neurons of SNpc from the neurotoxic damage and promote behavioural recovery. However, lentiviral delivery of GNDF to SNpc failed to prevent neurodegeneration in α-synuclein transgenic rat model of PD. Furthermore, recent clinical trials demonstrated divergent outcomes (Patel et al., 2005). GDNF has also been shown to induce resprouting of the lesioned nigrostriatal neuron system and thus may have beneficial effects on differentiated neurons in general.

Together with three other neurotrophic factors (neurturin, artemin, persephin) GNDF comprises a family of proteins, the so-called GDNF family ligands (GFLs). GFLs are distantly related to the transforming growth factor-beta superfamily, containing seven cysteine residues with the same relative spacing and acquiring similar conformation as the other members of this superfamily (Ibanez, 1998). The main signalling pathway of GFLs is mediated by RETreceptor tyrosine kinase (Sariola et al., 2003), which was initially discovered as a protooncogen (Takahashi, 2001).

2.6 Dopamine receptors

Only a decade ago the effects of dopamine were attributed to activation of two receptor subtypes, namely dopamine D1 and D2 receptors (Garau et al., 1978; Kebabian and Calne, 1979). However, in the early nineties, DNA cloning techniques led to novel, previously uncharacterized dopamine D3 (Sokoloff et al., 1990), D4 (Van Tol et al., 1991) and D5 (Tiberi et al., 1991; Sunahara et al., 1991) receptors. These receptors were not previously detected by pharmacological investigations and, today, the relevance of such diversity is subject of speculation. However, due to the similarities in structure, gene organization, signaling systems and pharmacology between the dopamine D1 and D5 subtypes and between the dopamine D2, D3, and D4 subtypes the dopamine receptors are still classified as D1-like and D2-like receptor subfamilies.

2.6.1 Dopamine D1-like receptor subfamily

The dopamine D1 and D5 receptor both consist of seven very similar membrane-spanning domains (Seeman and Van Tol, 1994; Lachowicz and Sibley, 1997). These domains are thought to play an important role in the pharmacological
binding profiles of the receptors and indeed the dopamine D1 and D5 receptor display a very similar profile (Gingrich and Caron, 1993). Gene transcripts of the dopamine D1 receptor are abundant in the brain; the highest densities of dopamine D1 receptors are found in the projection areas of the SNc and the ventral tegmental area (VTA), namely in the caudate putamen (CP), the nucleus accumbens (ACC) and the olfactory tubercle (OT), but significant amounts are also present in the neocortex, the pallidum and the amygdala (Mansour et al., 1990). Dopamine D5 receptors are less abundantly present in the brain; however, relatively high densities are found in the hippocampus, the hypothalamus and the parafascicular nucleus of the thalamus (Tiberi et al., 1991; Bergson et al., 1995).

2.6.2 Dopamine D2-like receptor subfamily

The dopamine D2, D3 and D4 receptor have somewhat more variety in the organization of their trans-membrane domains, which may contribute to considerable differences in binding characteristics of dopamine D2 receptor subtypes (Gingrich and Caron, 1993). A short and a long isoform of the dopamine D2 receptor, produced by alternative splicing of the same gene, exist. Like the dopamine D1 receptor, the dopamine D2 receptor is abundantly present and shows some similarities in distribution. High densities are present in the CP, the ACC, the OT and the SNc, while intermediate densities are found in the central nucleus of the amygdala, the lateral septum, the entorhinal cortex, the superior colliculus and the hippocampus (Boyson et al., 1986; Mansour et al., 1990). The D3 receptor is predominantly found in the OT and the shell of the ACC (Sokoloff et al., 1990). The density of dopamine D4 receptors is very low; however, moderate levels are present in the frontal cortex, the amygdala and, to a lesser extent, the striatum (Defagot and Antonelli, 1997).
2.7 Signal transduction mechanisms of dopamine

![Figure 2.3](image)

**Figure 2.3** Signal transduction mechanisms of dopamine.

Above Figure 2.3 gives a schematic representation of the signal transduction processes of the dopamine transmitter system. After the binding of dopamine to postsynaptic receptors, the coupling with G proteins leads to the transmission of the extra-cellular signal to intra-cellular sites. This intracellular site may be either a second messenger or an ion channel.

The majority of dopamine D1 receptors is coupled the adenylate cyclase (AC) second messenger system. Activation of the dopamine D1 receptor induces stimulation of the Gs protein that then stimulates AC to convert adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), in the presence of Mg$^{2+}$ ions (Zhou et al., 1990). The resulting increase in cAMP stimulates protein kinase A (PKA), which in turn stimulates the phosphorylation of a large variety of proteins, including Na$^+$ channels, Ca$^+$ channels, various receptors, Na/K ATP-ase, ß adrenoceptors and tyrosine hydroxylase (Walsh and Van Patten, 1994). Thus, the AC-coupled dopamine D1 receptor is intimately involved in ion fluxes, neurotransmitter release and gene transcription.

However, also other second messenger systems have been identified; among others, evidence is increasing that dopamine D1 receptors can activate the phosphoinositide second messenger system (Undie et al., 1994; Wang et al., 1995).
Stimulation of these receptors activates a G protein coupled to phospholipase C (PLC) that in turn hydrolyses the membrane-associated phosphatidylinositol 4, 5-bisphosphate (PIP2). PIP2 is converted into two second messengers, namely diacyl glycerol (DAG) and inositol triphosphate (IP3). DAG remains in the membrane and stimulates protein kinase C (PKC), while IP3 diffuses into the cytoplasm where it releases Ca\(^{2+}\) from the endoplasmatic reticulum, and activates Ca\(^{2+}\) dependent functions including the stimulation of PKC (Berridge and Irvine, 1989). Many neuronal proteins are substrate to PKC, including Na\(^+\) channels, Ca\(^{2+}\) channels K\(^+\) channels, GAP 43 and GABA-A, nicotinic and NMDA receptors.

Various second messenger systems participate in dopamine D2 receptor signal transduction, among which the AC transduction system. In the striatum, dopamine D2 receptor stimulation inhibits a Gi protein that inhibits AC to convert ATP into cAMP, and, thus, the coupling of dopamine D1 and dopamine D2 receptor to this transduction system is opposed. Moreover, dopamine D2 receptors affect the release of arachidonic acid from phospholipids (Tang et al., 1994) and influence ion channels; they increase the efflux of K that results in the hyperpolarization of neurons. In addition, the influx of Ca\(^{2+}\) is reduced by dopamine D2 receptor stimulation (Vallar and Meldolesi, 1989).

### 2.8 Connections of the midbrain dopamine neurons with brain areas involved in motor control

The fast majority of dopamine containing nerve cells is found in the midbrain. Several different forebrain projections from the midbrain dopaminergic cell groups are distinguished, including the mesostriatal, mesocortical and mesolimbic projections (Loughlin and Fallon, 1984). The nigrostriatal projection comprises the dorsal component of the mesostriatal system, which originates primarily from the dopamine cells of the SNc and, to a lesser extent, from the dopamine cells of the retrorubral area and the VTA (Beckstead et al., 1979). It ascends via the medial forebrain bundle and the internal capsule to innervate the CP and the globus pallidus (GP). Through this connection the SNc is functionally linked to a collection of brain areas, the basal ganglia. These areas are believed to have a predominant role in motor control. Moreover, studies showed that the basal ganglia play a definite role in cognitive behavior. The degeneration of this projection leads to a deficiency in dopamine in the
CP, viz. the primate analogue of the rodent dorsal striatum, and is critically involved in the pathology of PD. The type of motor and cognitive symptoms present in patients with Parkinson’s disease can be modeled by manipulating the dopamine function of the basal ganglia brain structures in rats, felines and non-human primates (van Oosten et al., 1998; Arts and Cools, 1999) which underlines the important role of these areas in the pathophysiology of PD.

The basal ganglia consist of five closely interlinked structures: the CP, the internal and external segment of the GP (GPi and GPe), the subthalamic nucleus (STN) and the substantia nigra, pars reticulate (SNr). Dopaminergic neurons from the SNc project onto GABA-ergic medium spiny neurons of the striatum. These neurons also receive massive glutamatergic input from the neocortex via the cortico-striatal pathway and from the cerebellum via the intralaminar nuclei of the thalamus. Within the basal ganglia, the information is processed via pathways from the medium spiny neurons of the striatum to the output structures of the basal ganglia, the SNr and the GPi. When movements are absent, the neurons in the SNr and GPi are tonically active, and their constant release of GABA produces a powerful inhibition of target cells in the ventral thalamic nuclei, superior colliculus and pendunculo pontine nucleus. The medium spiny neurons affect the activity of the SNr and GPi neurons through two pathways: a subclass of medium spiny neurons projects directly to the SNr and GPi whereas another subgroup projects to the output nuclei through an indirect route that includes the GPe and the STN. All medium spiny neurons form inhibitory synapses with the GPi and SNr that release GABA. Execution of movements involves an increased activity of the corticostriatal pathway exciting the medium spiny neurons, which leads to a rapid silencing of the inhibitory GPi and SNr neurons through the direct pathway. Consequently the thalamic, collicular and pontine neurons than exhibit a burst of activity. The glutamatergic stimulation of striatal neurons projecting to the indirect pathway tends to oppose this action: the striatum suppresses the activity of neurons in the GPe which releases the STN from inhibition and in turn excites the GPi and SNr. It has been proposed that the inhibitory effect of this subclass of striatal neurons serves a “smoothing” function on motor excitation (Smith et al., 1998).
The nigrostriatal dopamine projection exerts a net excitatory influence on striatal output neurons of the direct pathway, but a net inhibitory effect on the neurons giving rise to the indirect pathway. The excitatory effect is thought to be mediated by the stimulation of dopamine D1 receptors that are located primarily on the direct pathway neurons while the inhibitory effects are likely to be mediated through the stimulation of dopamine D2 receptors located mainly on the indirect pathway neurons. As a net result, the innervation of spiny neurons by nigral dopamine fibers releases the thalamic and brainstem nuclei from the tonic suppressing effects of the GPe and SNr. The increased activity of the latter nuclei increases their glutamatergic output to the various cortical areas involved in motor control. Thus, a loop exists between the cortex and the basal ganglia through the ventral thalamic nuclei, suggesting that the basal ganglia influence the output of the cortex to lower brainstem regions to execute movements. However, the direct projection of the SNr to brainstem areas, including the superior colliculus (Beckstead et al., 1979) and the pedunculo-pontine nucleus (Spann and Grofova, 1991) shows that the basal ganglia exert direct effects on motor control as well (Jaspers et al., 1989).

Despite the characterization of five dopamine receptor subtypes, dopamine receptors are still classified according to their signaling system and pharmacology into D1-like receptors and D2-like receptors. Moreover, the majority of the dopamine receptors in the CP complex that has lost its dopaminergic input in the parkinsonian brain, belong to either the dopamine D1 or the dopamine D2 subtype. The most important transduction system is the AC second messenger system, which is stimulated by dopamine D1 receptor stimulation through a Gs protein and inhibited by dopamine D2 receptor stimulation through a Gi protein. Dopamine plays a facilitating role in motor behavior at the level of the striatum. It modulates the regulatory role of cortical areas involved in motor behavior through the indirect and direct pathways in the basal ganglia and, in addition, exerts a direct regulatory role by directly innervating brainstem areas.

The use of mammalian animal models to study human locomotor disorders, such as those occurring in Parkinson’s disease, present some interesting challenges with respect to differing modes of locomotion in bipeds and quadrupeds. Rats, like all quadrupeds adopt several different gaits for locomoting overground, such as walking,
trotting, galloping, and hopping. They are able to compensate for locomotor disorders in ways that are not available to humans. Nevertheless, one common characteristic between bipedal gaits and many quadrupedal gaits is bilateral symmetry. Examination of locomotion overground in quadrupeds that are trotting, for example, reveals that a trot is comprised of alternating diagonal limb pairs in contact with the ground during each stride. This gait is symmetrical and alternating as is the bi-pedal walking gait. Although results of examinations of movement disorders in rats are not directly translatable to humans, valid and valuable comparisons can be made as a result of the conservation of the neural circuitry involved in control of locomotion and the components of symmetry in the gaits.

2.9 Animal Models of Parkinson’s disease

2.9.1 6-OHDA

The first animal model of PD to achieve dopaminergic cell death in the SNc involved use of the neurotoxin 6-hydroxydopamine (6-OHDA) in rats (Ungerstedt, 1968). 6-OHDA is a structural analog of dopamine. It is uptaken by dopaminergic and noradrenergic transporters (Luthman et al., 1989) and leads to cell death by non-apoptotic mechanisms (Jeon et al., 1995). Oxidative stress has long been recognized as a mechanism of 6-OHDA-induced cell death (Sachs and Jonsson, 1975). 6-OHDA is toxic to mitochondrial complex-I and promotes formation of superoxide free radicals and quinones (Bove et al., 2005).

Because 6-OHDA does not cross the blood-brain barrier, researchers have infused it stereotaxically into the brain in several ways, including intraventricular, intracisternal, and intracerebral, both intraventricular and intracisternal injection of 6-OHDA causes a bilateral catecholaminergic lesion that is apparent within several hours (Bove et al., 2005). Animals given severe bilateral lesions often display aphagia, adipsia, and seizures (Ungerstedt, 1971), which limits the usefulness of bilateral lesions of 6-OHDA for PD model research. Animals given unilateral intracerebral injections, on the other hand, care for themselves adequately and are widely used for research. Common sites of intracerebral injection include the SN, medial forebrain bundle, and striatum (Schober, 2004).
Usually, dopamine content in the striatum is reduced by more than 97% after this lesion (Schmidt, R. H. et al., 1983; Rioux, L. et al., 1991; Kirik, D. et al., 1998). The expression of receptors was shown to be upregulated in the lesioned caudate-putamen (Gagnon, C. et al., 1991; Cadet, J. L. et al., 1992; Dawson, T. M. et al., 1991). Quantification of drug-induced rotations was later used as a simple behavioural test for estimation of the degree of the produced lesion (Ungerstedt, U. et al., 1970). Typically, two drugs are used for induction of the rotational behaviour in unilaterally lesioned animals: amphetamine and apomorphine. Amphetamine acts as an agonist of dopamine inducing the fast and almost complete release of the neuromediator dopamine from the presynaptic terminals (Sulzer et al., 2005). Apomorphine, a short-acting dopamine D1 and D2 receptor agonist, functions postsynaptically (Picada et al., 2005). An animal subjected to the injection of either of the drugs will rotate away from the site of a greater activity. This can be explained by the anatomical structure of the mammalian CNS, particularly the fact of crossing of the afferent tracts to the contralateral side at the level of either brainstem or spinal cord. However, due to the difference in mechanisms of action of the drugs, the rat exposed to amphetamine will rotate ipsilaterally, while the apomorphine-injected rat will exhibit contralateral to the lesion turns. Drug-induced rotational behaviour is often used to select the animals with complete lesions, since only those rats with complete degeneration of the nigrostriatal projections will exhibit robust turning behaviour induced by the drug.

2.9.2 MPTP and MPP⁺

The discovery of the neurotoxic effects of 1-methyl-4-phenyl-1, 2, 3, 6 tetrahydropyridine (MPTP) provided compelling evidence that environmental agents contribute to PD. Drug users who injected (Davis et al., 1979; Langston et al., 1983) 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP), a synthetic opiate analog of meperidine and was contaminated with MPTP, subsequently developed a syndrome clinically similar to PD. These patients initially responded well to L-dopa therapy and later experienced dyskinesias and on-off fluctuations similar to idiopathic PD patients (Ballard et al., 1985).

MPTP is highly lipophilic and readily crosses the blood-brain barrier (Shimohama et al., 2003). It is converted to 1-methyl-4-phenylpyridinium (MPP⁺) by monoamine oxidase B (MAO-B) in astrocytes and serotonergic neurons, and
transported into dopaminergic neurons by the dopamine transporter (DAT). Inside dopaminergic neurons, MPP+ inhibits mitochondrial complex-I (Nicklas et al., 1987), interrupting the flow of electrons in the mitochondrial electron transport chain and promoting production of reactive oxygen species, which trigger the process of apoptosis (Fiskum et al., 2003).

MPTP produces inclusions that resemble immature Lewy bodies (Forno et al., 1988; Kowall et al., 2000). Similar inclusion bodies have been reported in transgenic mice (Song et al., 2004), although others have reported no inclusion bodies (Shimoji et al., 2005). The MPP+ rat model of PD involves unilateral infusion of MPP+ into the nigrostriatal bundle, striatum, or substantia nigra. Because MPP+ is a charged particle that has not been shown to cross the blood-brain barrier, whereas MPTP is known to readily cross the blood-brain barrier, the unilateral MPP+ model is considered to be distinctly safer than MPTP models (Przedborski et al., 2001).

### 2.9.3 Paraquat

Paraquat (N,N'-dimethyl-4-4'-bipiridinium), a pesticide that is structurally very similar to MPP+, has been shown in mice to cross the blood-brain barrier, despite its charge (Brooks et al., 1999). Paraquat appears to cross the blood-brain barrier via L-neutral amino acid transporters, because pretreatment with L-valine or L-phenylalanine completely blocks neurodegeneration (McCormack et al., 2002). Mice that are chronically exposed to paraquat develop SNC dopaminergic neuron degeneration and a-synuclein-containing inclusions (Manning-Bog et al., 2002). The mechanism of paraquat-induced cell death involves reactive oxygen species (ROS) produced by reduction-oxidation cycling (Przedborski and Ischiropoulos, 2005).

### 2.9.4 Rotenone

Chronic systemic administration of rotenone has been shown to reproduce many features of PD in rats, including nigrostriatal dopaminergic degeneration, hypokinesia, rigidity, and cytoplasmic inclusions that contain ubiquitin and a-synuclein (Betarbet et al., 2000). However, high variability has been observed in the severity of dopaminergic cell damage caused by chronic rotenone injection (Zhu et al., 2004). Rotenone is highly lipophilic and thus quickly passes the blood-brain barrier, where it inhibits NADH-ubiquinone reductase in mitochondrial complex I, leading to cell death (Schuler & Casida, 2001).
2.9.5 Transgenetic model

Transgenic animals express a gene of interest foreign to their own DNA. To date, the two major transgenetic animal models are mouse α-synuclein overexpression (Masliah et al., 2000; Lee et al., 2002) and drosophila α-synuclein overexpression (Feany and Bender, 2000). In some of these transgenic models, α-synuclein expression leads to neurodegeneration that involves the dopaminergic system. These transgenic animals may actually model synucleinopathies, a group of neurodegenerative disorders where α-synuclein pathology is a common theme, rather than PD.

2.10 Neurochemical Assay

Tyrosine Hydroxylase (TH) Immunocytochemistry (Quantitative Morphology) is a standard conjugated secondary antibody method is used to mark TH (tyrosine hydroxylase) immunoreactive neurons in the substantia nigra pars compacta. These neurons are counted, on both right and left sides, using standard stereological techniques. Labeled cells are considered neurons if they possess at least two but no more than six processes. Counts of neurons expressing detectable levels of TH activity are a measure of functionality of substantia nigra DA neurons that survive the lesion.

2.11 Chemical information of Dopamine HCl

Synonymous names :- 2-(3,4-Dihydroxyphenyl) ethylamine hydrochloride or 3-Hydroxytyramine hydrochloride

Structural formula :-

![Structural formula of Dopamine HCl]

Empirical formula :- C₈H₁₁NO₂ . HCl

Molecular weight :- 189.65 g/mol

Melting Point :- 245 °C

The formation of dipeptide involves the condensation of the carboxyl group of one amino acid with the amino group of another. Amides can be readily synthesized
from amines and acid chlorides. However, this method does not work well for the synthesis of peptide chains because many amino acids have additional functional groups that also react with the acid chloride. Thus, the reaction may give a mixture of various products. To solve this problem, protecting groups are added to the amino acids to protect the reactive functional groups before the reaction is run. With the reactive groups protected, the reaction then forms the desired amide bond. When choosing protecting groups, biochemists look for groups that are stable in typical reaction conditions and that are easily removed later without affecting the amide bond. Two groups frequently used to protect the carboxylic acid functional group are methyl esters and benzyl esters. Both of these esters usually allow ready regeneration of the carboxylic acid (Furniss, 1989).

When removing the protection from the carboxylic acid functional group, both benzyl and methyl esters readily hydrolyze in aqueous base. An alternate way to remove the benzyl ester is through hydrogenation which cleaves the weak benzylic C—O bond. The reaction conditions that form the esters or regenerate the amino acid usually do not affect the amide groups of a peptide chain. Amino acid N- gives imides with phthalic anhydride. Amino acid N- is especially easy with cyclic anhydrides, which produce cyclic imides (March, 1992).

2.12 Mechanism of DCC Coupling

![Dicyclohexylcarbodiimide (DCC)]

The actual formation of the peptide bond is accomplished by a reaction involving an amino acid with a protected carboxylic acid, another amino acid with a protected amine, and dicyclohexylcarbodiimide (DCC). DCC converts the unprotected carboxylic acid to an intermediate with chemical properties similar to an acid anhydride. Thus, the carbonyl carbon of the carboxylic acid is much more reactive towards nucleophilic substitution than it would be otherwise (Daley, 2005).
Tremendous progress has been made in the treatment of numerous brain diseases in the past few decades. Unfortunately, many cerebral maladies, including infections, cancer and neurotransmitter imbalance are often refractory to chemotherapeutic intervention, making these diseases pernicious and often fatal. One of the reasons for this is the inability of many drugs to enter brain tissue in pharmacologically relevant concentrations.

The basis of this impermeability is the blood-brain barrier (BBB), which acts as a functional barricade to separate the brain from the systemic circulation. Structurally, the component endothelial cells of the CNS capillaries are joined tightly together, precluding intercellular bulk transport. This architecture forces compounds to diffuse directly through the cell membranes if they are to gain access to the brain parenchyma, and, as the cell membranes are phospholipoidal in nature. In the periphery, capillaries are leaky, allowing nonspecific intercellular transport. Brain
capillaries are also characterized by a high concentration of various enzymes that help to maintain the delicate cerebral microenvironment by preventing the uptake of blood-borne neurotransmitters and other substances.

General methods for improving drug flux into the brain would therefore be useful. Brain uptake of compounds can be improved by derivatization of the drug molecules via prodrug formation. A prodrug is a pharmacologically inactive compound that results from transient chemical modification of a biologically active species. The chemical change is designed to improve some deficient physicochemical property of the drug such as membrane permeability or water solubility. After administration the prodrug by virtue of its improved characteristics is brought closer to the receptor site for longer periods of time where it can convert to the active species.

Prodrugs usually require a single activating step. To improve the entry of a hydroxy, amino, or carboxylic acid-containing drug, esterification or amidation may be performed. This greatly enhances the lipid solubility of the drug, and as a result the drug can better enter the brain parenchyma. Once in the CNS, hydrolysis of the lipophilicity-modifying group will release the active compound.

Some of the limitations associated with the prodrug approach are derived from the fact that only one chemical conversion occurs in the activation of the compound. In many circumstances, multiple, facile conversions may not only lead to selectivity in delivery but may also act to decrease the toxicity of a drug, as well as sustain its action. A chemical delivery system (CDS) is defined as a biologically inert molecule that requires several steps in its conversion to the active drug and that enhances drug delivery to a particular organ or site. In designing a CDS for the CNS the unique architecture of the BBB was exploited. As with a prodrug, a CDS should be sufficiently lipophilic to allow for brain uptake. Subsequent to this step, the molecule should undergo an enzymatic or other conversion to promote retention within the CNS but at the same time to accelerate peripheral elimination of the entity. Finally, the intermediate should degrade, releasing the active compound in a sustained manner. Upon systemic administration the CDS can partition into several body compartments due to its enhanced lipophilicity, some of which are inaccessible to the unmanipulated compound.
The conjugate that is trapped behind the BBB can then slowly hydrolyze to give the active species in a slow and sustained manner. By the system design, concentrations of the active drug are very low in the periphery reducing systemic, dose-related toxicities. In addition, the drug in the CNS is present mostly in the form of an inactive conjugate, thus lowering any central toxicity. This approach should allow for a more potent compound in that a larger portion of the administered dose is shunted to its site of action. In addition this system should allow for increased dosing interval. Since it was first proposed in 1978, the brain-targeting CDS has been extensively applied to various pharmacologically active agents.

Application of the CDS in the case of dopamine was considered particularly apropos since the parent compound does not cross the BBB and as such is not useful in replacement therapy. L-Dopa, a prodrug of dopamine, has been used clinically for treating Parkinsonism, but this material exerts peripheral toxicities that can be dose limiting.
Figure 2.5 Chemical delivery system of dopamine.

A CDS for dopamine was therefore designed and synthesized by first condensing nicotinic acid and dopamine hydrobromide to yield the catechol nicotinamide derivative. Subsequent pivaloylation gave the bis ester, which was then quaternized and reduced to give rise to the protected dopamine delivery system (DA-CDS) in the above Figure 2.5.
In *vitro* studies indicated that the necessary transformation required to liberate dopamine occurred, meaning that the DA-CDS was converted to the corresponding quaternary salt (P-DA-Q⁺), which further underwent sequential ester hydrolysis to give the 1-methylnicotinamide of dopamine (DA-Q⁺), the ultimate dopamine precursor or prodrug. Systemic administration of the DA-CDS to rats caused high levels of the DA-Q⁺ to accumulate in the CNS while blood levels were shown to be rapidly cleared. This uncoupling of brain and blood concentration of the administered compounds and its metabolites is a characteristic finding of the CNS approach.

Initial tests used to show dopamine release included such pharmacologic responses as the ability of dopamine to decrease prolactin secretion. This action is manifested by the ability of this neurotransmitter to interact with lactophores in the anterior pituitary gland. In testing the DA-CDS, male rats were primed with beta-estradiol to elevate serum prolactin. Administration of DA-CDS to these animals resulted in suppression of serum prolactin by approximately 80% for over 12 hours. In contrast, the polar dopamine precursor P-DA-Q⁺ significantly suppressed prolactin levels only at 30 minutes, after which serum prolactin returned to control values. These studies therefore, suggest that dopamine is being slowly released from DA-Q⁺ after brain sequestration subsequent to DA-CDS administration. The facts that CDS in and of itself not active and that hydrolysis was necessary for activity were shown using isolated anterior pituitary glands. Dopamine at a concentration of 200 nM significantly reduced the rate of prolactin secretion while the DA-CDS had no effect, suggesting it possesses a much lower binding affinity for the pituitary lactophores.

More direct evidence for dopamine release was observed in rats pretreated with the aromatic amino acid decarboxylase inhibitor m-hydroxybenzylhydrazine. Animals administered the DA-CDS demonstrated a dramatic increase of dopamine in the hypothalamus (400% to 500%) and significant increases (17% to 20%) in the striatum. In addition, dopamine metabolites, including dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were also substantially increased in hypothalamus and striatum after DA-CDS administration (Kozikowski, 1993).
2.13 Amino acids analogues as building blocks for peptidomimetics

A number of building blocks not belonging to the 20 proteinogenic amino acids have been devised as replacement for amino acids in a natural peptide sequence. Such units can stabilize and/or induce secondary structure motives in peptides, due to the lack of side chains (glycine, beta-alanine), due to their chirality (D-amino acids), or due to the prolongation of the peptide back-bone (beta-amino acids) or to some constraining features which can rigidify the peptide structure (α,α-dialkyl and cyclic amino acids) (Sidney, 2001; Weinstein, 1983).

Peptides are complex molecules and each sequence is unique with regard to its chemical and physical properties. While some peptides are difficult to synthesize, many peptides are relatively straightforward to synthesize but may still be difficult to purify after synthesis. A common problem with many peptides is insolubility in aqueous solution (Milton, 1990).

Invention relates to dipeptide derivatives with central nervous system activity. The main obstacle to the practical use of many biologically active peptides is their brief period of action which is partly due to their inactivation by proteolytic enzymes. An example of such a peptide is the tripeptide which is the factor inhibiting release of the melanocyte stimulating hormone (MIF or MRIH). The tripeptide was isolated from bovine hypothalamic tissue (Nair et al., 1971) and its structure was established as the C-terminal tripeptide of oxytocin H-L-prolyl-L-leucyl-glycinamide. This tripeptide was shown to exert an action on the central nervous system (CNS). The tripeptide potentiates the behavioral effects of (3, 4-dihydroxyphenyl)-L-alanine (L-DOPA) (Plotnikoff et al., 1971; Friedman et al., 1973). The tripeptide antagonizes the effects of oxotremorin (Plotnikoff, 1972) and reverses the sedative effects of deserpidine in mice and monkeys (Plotnikoff, 1972). On the basis of the above biological activities it may be suggested that the tripeptide H-Pro-Leu-Gly-NH₂ could be useful in the treatment of patients suffering from depression and parkinsonism (Schally et al., 1973).

The dipeptide derivatives produced by the process of this invention, as well as their corresponding therapeutically acceptable salts, are useful because they show the
pharmacological activities upon the CNS of mammals possessed by the natural tripeptide H-L-prolyl-L-leucylglycinamide. For example, the compounds of this invention potentiate the effects of L-DOPA (Olotnikoff et al., 1971). The dipeptide derivatives also antagonize fluphenazine induced catalepsy in rats, an animal model particularly suitable for screening compounds useful in the management of Parkinson like disorders, and they cause reversal of the sedative effect of deserpidine, an animal model of depression. The dipeptide derivatives of this invention have a prolonged duration of action and are useful for treating or managing central nervous system disorders, especially Parkinsonism and mental depression, in mammals. When a dipeptide of this invention or a salt thereof is employed for such treatment or management, it is administered systemically, preferably parenterally, in combination with a pharmaceutically acceptable liquid or solid carrier. The dipeptides have a low order of toxicity (Sestanj and Hans, 1978).

2.14 Dopaminergic Treatments for Parkinson’s disease

Available pharmacotherapy for PD continues to be palliative or symptomatic, involving partial compensation the DA deficiency in neostriatum, usually treated by direct stimulation of DA receptors, or by enhancing its synthesis or decreasing its catabolism. No treatment has been proved to stop or even slow the progressive neurodegeneration in PD. However, evidence of a lesser rate of loss of DA neurons has recently been reported during 4 years of treatment with the direct DA agonist pramipexole compared to L-dopa.

![Pramipexole](image-url)
2.15 L-Dopa Metabolism and Therapy

Nearly 40 years after its introduction, levodopa or L-dopa remains an effective pharmacotherapy in PD. Development of L-dopa as a therapeutic agent in PD is a rare example of a rationally predicted and logically pursued clinical treatment in a neurological disorder, based on neurochemical pathology and basic pharmacological theory. The effectiveness of L-dopa treatment requires its penetration into the central nervous system (CNS) and local decarboxylation to DA. L-Dopa is normally a trace intermediary metabolite in the biosynthesis of catecholamines, formed from L-tyrosine in a rate-limiting hydroxylation step by tyrosine hydroxylase; a phosphorylation activated cytoplasmic mono-oxygenase. L-Dopa is readily decarboxyated by the cytoplasmic enzyme L-aromatic amino acid decarboxylase ("dopa decarboxylase") to form DA.

Most exogenous L-dopa is rapidly decarboxyated to DA in peripheral tissues, including liver, heart, lung, and kidney. Because only about 1% of an administered dose reaches the brain, L-dopa, by itself, has very limited dose effectiveness. In humans, appreciable quantities of L-dopa enter the brain only when administered by itself in doses (3-6 g daily) high enough to overcome losses caused by peripheral metabolism. Inhibition of peripheral decarboxylase activity by co-administration of L-dopa with a hydrophilic, peripheral decarboxylase inhibitor such as carbidopa (Syndopa, Sinemet and Sinemet-SR) or benserazide markedly increases the proportion of L-dopa that reaches the brain, where it can be converted to DA by widely available aromatic amino acid decarboxylase and replace its deficiency associated with PD (Abraham, 2003).

Adverse reactions to levodopa therapy can be grouped into two classes. In the first class are those that occur early in therapy and to which tolerance develops; the most common of these are the gastrointestinal symptoms of anorexia, nausea, and vomiting. These side effects are thought to be caused by direct stimulation of the chemoreceptor trigger zone of the area postrema in the medulla oblongata by the newly formed dopamine.
A second class of adverse side effects all increase with increasing duration of levodopa therapy and result from the central stimulation of dopamine receptors. The most common and serious of these side effects are abnormal choreiform movements of the limbs, hands, trunk, and tongue. These dyskinesias eventually occur in 40 to 90 percent of patients receiving long-term high-dosage levodopa therapy. The mechanism by which these abnormal movements are produced appears to be related to the presence of hypersensitive dopamine receptors. It is not clear whether this occurs as a result of denervation supersensitivity or from continuous stimulation by dopamine for long periods of time.

Serious mental disturbances occur in 10 to 15 percent of patients receiving levodopa. Although these disturbances may reflect underlying pathology, they are likely to be drug-induced. Patients on long-term therapy with levodopa can experience psychotic episodes (i.e., vivid dreams, delusions, and visual hallucinations). If such episodes occur, the dose of levodopa or other dopaminergic drugs should be reduced. If this is unsuccessful, it may be necessary to put the patient on a limited “drug holiday” where all drugs are withdrawn for 1 day, a week or, alternatively, if the psychosis is very severe, on a more prolonged drug holiday in which the drugs are withdrawn for at least 5 days. This latter approach is less desirable since the patient could deteriorate to a severe state of immobility.

Levodopa therapy in Parkinsonism is frequently characterized by a high day-to-day and within-day variation in effectiveness. However in a few patients, this phenomenon becomes marked and is called the “on-off phenomenon”. In a matter of minutes, a patient enjoying normal or near-normal mobility may suddenly develop a severe degree of Parkinsonism. The tremor reappears, normal postural reflexes disappear, and bradykinesia suddenly develops; such symptoms may persist from 30 min to 3 to 4 hr. These on-off cycles may occur anywhere from once a day to 10 or more times daily during the waking hours. This problem usually develops after 2 years of levodopa treatment. There is no completely satisfactory explanation for it, although the abrupt nature of the oscillation suggests an imbalance in physiological regulatory mechanisms. Although the cycles are not clearly related to blood levels of either levodopa or dopamine, the on-off phenomenon usually occurs when the blood
levels are in the lower region of the therapeutic range (Craig and Stitzel, 1997).

**2.16 Clinically Used Dopamine D₂ Receptor Agonists**

The nigrostriatal neurodegeneration underlying PD reduces the number of striatal nerve terminals available to decarboxylate L-dopa to DA, but also increases sensitivity of DA receptors as well as loss of the inactivation of DA by neuronal reuptake at DA transporters virtually expressed only by DA neurons and their terminals. Drugs that act directly to stimulate DA receptors do not require functioning DA nerve terminals or endogenous synthesis of DA, and can be particularly useful in managing late-stage PD. Several agents with direct DA-agonist activity have been used in the treatment; all are primarily agonists or partial agonists of the D₂ family of DA receptors. No agents with selective D₁ agonist activity, or well-balanced D₁ and D₂ agonist actions have been developed for clinical application, though apomorphine has some D₁ as well as potent D₂ agonist effects.

![Apomorphine](image-url)

Bromocriptine and other D₂ partial-agonist ergolines act as D₂ agonists with antiparkinson, and perhaps mood-elevating, effects. This agonism evidently reflects the supersensitized status of denervated DA receptors in PD. Pergolide shows greater agonist effects at both D₂- and D₁-type DA receptors than doe’s bromocriptine. Several additional experimental ergolines have been developed with a range of D₂ agonist or partial-agonist actions, including cabergoline, lergotrile, Pergolide, and lisuride.
Currently, ropinirole and pramipexole are among the most commonly prescribed direct DA agonists for PD in the United States. They were introduced primarily for use in advanced stages of PD to limit fluctuations in response to L-dopa therapy and as a "rescue" therapy when L-dopa became less effective (Abraham, 2003).

Recent advances in the fields of pharmacology and molecular biology have led to a greater understanding of disease processes, allowing development of new classes of therapeutic agents that can interact with specific intracellular and extracellular targets. Several drugs, peptides, biological response modifiers, and monoclonal antibodies are available and have proven of value:

(a) In inhibiting a variety of malignant and infectious diseases;
(b) In ameliorating neurotransmitter, enzyme, or growth imbalances in culture systems;
(c) In animal models using direct intracranial administration.

However, therapeutic efficacy in vivo, particularly with regard to the central nervous system (CNS), frequently is diminished or prevented by the inability of the agent to reach and maintain active concentrations in the brain for an appropriate length of time. Frequently, the molecule is too large or has polar functional groups, so that its access to the brain target organ is limited by the blood-brain barrier (BBB) (Rapoport, 2004).

Chemically conjugated brain specific drug delivery system represents a rational drug design approach that is exploited for delivery of the drug to the target site of action (brain) as well as reduce the toxicity and increase the treatment efficacy (Drion, 1996). It is known that drug delivery to brain is quite difficult due to presence of the blood brain barrier (BBB) (Singour and Shrivastava, 2004).

The blood-brain barrier (BBB) used to be considered impermeable to polypeptides. However, this view has evolved rapidly over the past two decades. Not only do polypeptides have the potential to serve as carriers for selective therapeutic agents, but they themselves may directly cross the BBB after delivery into the bloodstream to become potential treatments for a variety of CNS disorders, including neurodegeneration, autoimmune diseases, stroke, depression, and obesity. The interactions of polypeptides with the BBB can take many forms, such as simple diffusion, saturable transport, or facilitation of entry of another peptide or protein. In some instances, interactions in the blood compartment (outside the BBB) or within the endothelial cells (at the BBB level) can significantly impede the passage of polypeptides across the BBB. Review of different aspects of interactions between peptides/proteins and the BBB that affect their delivery as potential drugs in their natural form, and discuss recent advances in the cell biology of polypeptide transport across the BBB. Better understanding of the BBB was provided insight and direction for future research in the treatment of CNS disorders (Pan and Kastin, 2004).

Parkinson’s disease is one of the most likely neurological disorders to be fully treatable by drugs and new therapeutic modalities. The age-dependent and multifactorial nature of its pathogenesis allows for many strategies of intervention and
repair. Most data indicate that the selectively vulnerable dopaminergic neurons in the substantia nigra of patients that have developed Parkinson’s disease can be modified by protective and reparative therapies. First, the oxidative stress, protein abnormalities, and cellular inclusions typically seen could be dealt with by antioxidants, trophic factors, and proteolytic enhancements. Secondly, if the delay of degeneration is not sufficient, then immature dopamine neurons can be placed in the parkinsonian brain by transplantation. Such neurons can be derived from stem cell sources or even stimulated to repair from endogenous stem cells. Novel molecular and cellular treatments provide new tools to prevent and alleviate Parkinson’s disease. Cell replacement therapy seeks to replace the loss in synaptic signaling cause by the neuronal degeneration. It has been shown that fetal ventral mesencephalic neurons transplanted to the caudate putamen of PD patients can significantly reduce the need of L-DOPA treatment and improve symptoms (Freed et al., 2002).

The brain is a dynamic and highly regulated organ compartmentalized by the BBB (cerebral endothelial cells), and the blood-CSF barrier (CP epithelial cells). Brain parenchymal cells (i.e., neuroglia and neurons) exist within this highly regulated environment and function in intimate interplay with one another. Each brain compartment possesses a specific and selective set of metabolic enzymes, receptor proteins, and secretory factors that serve to maintain the homeostatic environment that is necessary for normal function within that compartment. In addition, the localization and expression of various putative drug transporters in these barriers play a critical role in the influx/efflux of numerous xenobiotics and has an important impact on the overall pharmacokinetic/pharmacodynamic profile of drugs in the brain (i.e., distribution, pharmacological response, drug-drug interactions). Novel localization and functional expression of standard transporters (i.e., NT) as well as the efflux transporters (P-gp and MRP) in brain parenchyma suggest a reconsideration of the present conceptualization of brain barriers as it relates to drug transport. The cellular membranes of parenchyma cells, such as microglia and astrocytes, also act as barriers to drug permeability and express transporters whose properties appear similar to those localized to the conventional brain barriers (i.e., cerebral endothelial cells and CP epithelial cells) (Lee et al., 2005).
The BBB plays an important role in brain homeostasis and a number of transport systems are present that enable substances to enter the brain. Specific transport systems have been reported for amino acids, glucose, and iron transferrin. Several saturable transport systems have also been reported for peptides, both influx and efflux (Egleton and Davis, 2005).

2.17 Blood brain barrier

![Blood brain barrier](image)

**Figure 2.6** Blood brain barrier.

Blood brain barrier discovered by Paul Ehrlich in 1929, is a physical metabolic and immunological barrier that protects the brain microenvironment from toxins. BBB develops in the first trimester of fetal life. It occupies 2% of the body space and utilizes 15% of the body glucose. The BBB is formed by the endothelium that lines the cerebral microvessels of the brain. Special cells called astrocytes are present in BBB and their main function is regulation of enzyme expression in the endothelium. The other biochemical entities present in BBB include enzymes like MAO A and B, COMT; hormones like adrenalins, dopamine, histamine, PG. and bradykinins and proteins such as P-glycoproteins. Glucose, oxygen, carbon dioxide, water, and most lipid soluble substances, such as alcohol, caffeine, nicotine, heroin, and most anesthetics, pass rapidly from the circulating blood into brain cells. Other substances, such as creatinine, urea, and most ions, for example, Na⁺, K⁺, and Cl⁻ enter quite slowly. The different rates of passage of certain materials from the blood into most parts of the brain depend on the blood-brain- barrier (Tortora *et al*., 1993).
The blood-brain barrier (BBB) is a vital element in the regulation of the constancy of the internal environment of the brain. The composition of the extracellular fluid of the brain is controlled within very precise limits, largely independently of the composition of the circulating blood, to provide a stable environment in which the integrative neuronal functions of the brain can optimally take place. The blood-brain barrier is formed at the level of the endothelial cells of the cerebral capillaries. These cells are characterised by having tight continuous circumferential junctions between the cells of the capillaries thus abolishing any aqueous paracellular pathways between the cells. The endothelium is thus characterised by exhibiting a high transendothelial electrical resistance in the region of 1500-2000 Ω cm². The presence of the tight junctions and the lack of aqueous pathways between cells greatly restrict the movement of polar solutes across the cerebral endothelium.

Some regions within the central nervous system (CNS) lack a BBB and the capillaries are fenestrated allowing the free movement of solutes between the blood and the surrounding interstitial fluid. These areas are collectively termed the circumventricular organs (CVOs) and comprise the choroid plexus. Because of the presence of the BBB a number of special transport mechanisms are required to be present in the cerebral endothelial cells to ensure that the brain receives an adequate supply of nutrients.

2.17.1 BBB transport mechanisms

Amino acids are transported across the BBB on a variety of transporters. Large neutral amino acids, such as tyrosine, phenylalanine, leucine, isoleucine, valine, histidine and methionine are transported to the brain by an energy dependent transporter termed system –L which is present at the luminal and abluminal membranes and is principally directed from blood to endothelial cell and from endothelial cell into brain.

System-A, transporting principally glycine and proline is present on the abluminal membrane of the endothelial cells and directed out of the brain. A transporter for the dicarboxylic amino acids, glutamic and aspartic acid is also present in the BBB and is directed out of the brain. Both system-A and system-ASC are sodium- as well as energy-dependent. A number of specific receptors for solutes also exists both on the luminal and abluminal surface of the endothelial cells. These
receptors may be linked to second messengers such as cAMP or may modulate the activity of channels or transporters in the BBB. There may also be transporters in the cell membrane for solutes such as small peptides (David, 1996).

The BBB also has a physiological and biochemical dimension in that endothelial cells have a high density of mitochondria compared with other endothelia presumably reflecting a high level of oxidative ATP production. In addition the BBB is the site of a high level of enzyme activity directed towards the inactivation of centrally active bloodborne solutes and toxins (Audus et al., 1996).

**Figure 2.7** Interaction of solutes with the blood-brain barrier.

Solute both in blood and in the brain extracellular fluid may interact with the BBB in a variety of ways.

The intrinsic membrane protein P-glycoprotein sometimes referred to as multidrug resistance protein (mdr protein) is also present in the luminal plasma membrane of the endothelial cells constituting blood-brain barrier. This is an efflux pump which appears to be a constituent part of the BBB and transports a wide range of structurally unrelated substances out of cells. Pgp is an ATP-dependent pump and is a member of a family of intrinsic membrane proteins which are normally expressed
at the BBB, the intestine, the liver and the kidney. It acts to maintain the intracellular levels of cytotoxic drugs below a toxic level and thus frustrates repeated cancer chemotherapy. Its constitutive role in the normal BBB is inferred to be a protective one reducing the entry of lipophilic and neurotoxic substances into the CNS. Various physical factors influencing brain uptake have been incorporated into a predictive equation for brain uptake (Abraham et al., 1994).

To maximize the brain uptake and the bioavailability of substance to the brain a number of molecular manipulations may be carried out to alter the properties of a substance: these may be (Begley, 1996):

1. Increasing the plasma stability and hence plasma half life.
2. Improving lipid solubility.
3. Enhancing or maintaining reactivity with existing BBB transport mechanisms.
4. Retaining central nervous activity.
5. Increasing the stability in brain extracellular fluid and reducing reactivity with efflux transport mechanisms in the CNS.
Table 2.1 Main types of transport systems for targeted delivery of peptides across the BBB.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Characteristics</th>
<th>Example</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Diffusion</td>
<td>Flux down an electrochemical gradient</td>
<td>CTAP (transcellular)</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td>Energy independent</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flux proportional to concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rate independent of action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B Facilitated</td>
<td>Carrier mediated</td>
<td>Biphillin via LNAA</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Flux is saturated by increasing concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Competitive substrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flux may be asymmetric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active carrier mediated</td>
<td>Flux can be against electrochemical gradient</td>
<td>β-Endorphin efflux via P-glycoprotein</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Energy dependent (directly or indirectly)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Substrate specificity, saturation, competition</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flux rate is asymmetric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Endocytosis</td>
<td>Invagination of plasma membrane to form an internalized membrane vesicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Usually energy dependent</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flux against gradient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid phase</td>
<td>Soluble molecules internalized with the vesicle volume</td>
<td>Lucifer yellow</td>
<td>145,146</td>
</tr>
<tr>
<td></td>
<td>Nonsaturable, nonspecific dependent on solubility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adsorptive</td>
<td>Solute nonspecifically adsorbs to cell surface proteins/glycoproteins</td>
<td>gp-120</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>Can be saturable and show competition, high capacity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptor mediated</td>
<td>Highly specific</td>
<td>Insulin</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>High affinity, saturable, low capacity</td>
<td>Vectors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asymmetric</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CTAP = d-Phe-Cys-Tyr-Arg-Thr-Per, Thr-NH$_2$. 
Figure 2.8 The main types of transport systems that can be targeted for BBB delivery.

A = free diffusion; B = carrier-mediated transport; C = receptor-mediated transport

Water soluble peptides are normally not transported through the brain capillary wall, i.e. the blood-brain barrier (BBB). Chimeric peptides may be transportable through the BBB and are formed by the covalent coupling of a nontransportable peptide, e.g. beta-endorphin, to a transportable peptide vector, e.g. cationized albumin, using disulfide-based coupling reagents such as N-succinimidyl 3-[2-pyridyldithio(propionate)] (SPDP). The transcytosis of peptide into brain parenchyma, as opposed to vascular sequestration of blood-borne peptide, was quantified using an internal carotid artery perfusion/capillary depletion method. It is shown that beta-endorphin is not transported through the BBB, but is rapidly cleaved to free tyrosine via capillary peptidase. Therefore, chimeric peptide was prepared using [D-Ala2]beta-endorphin (DABE), owing to the resistance of this analogue to peptidase degradation. The DABE-cationized albumin chimeric peptide is shown to enter brain parenchyma at a rate comparable to that reported previously for unconjugated cationized albumin. When the DABE-cationized albumin chimeric peptide was incubated with rat brain homogenate at 37 °C, the free DABE was liberated from the cationized albumin conjugate prior to its subsequent degradation into free tyrosine. Approximately 50% of the chimeric peptide was cleaved within 60 sec of incubation at 37 °C. These studies demonstrate that beta-endorphin is not transported through the BBB in its unconjugated form, DABE-cationized albumin chimeric peptide is transported through the BBB into brain parenchyma at a rate comparable to the unconjugated cationized albumin, and brain contains the necessary disulfide reductases for rapid cleavage of the chimeric peptide into free beta-endorphin and this cleavage occurs
before degradation of the DABE into tyrosine (Pardridge et al., 1990). To overcome the limited access of drugs to the brain, various strategies have been applied to direct central nervous system (CNS) drugs into the brain (Temsamani et al., 2003). Most of these methods are invasive, such as surgical implantation of an intraventricular catheter followed by drug infusion into the ventricular compartment, transient opening of the tight junctions by the intracarotid infusion of a hypertonic solution (Chamberlain et al., 1993), or intracarotid arterial infusion of vasoactive substances such as bradykinin or bradykinin analogs (Bartus et al., 2003).

Alternative, noninvasive methods that exploit the formation of chimeric peptide or protein-drug conjugates as carriers have also been developed. One such method relies on the presence of specific receptor-mediated transport systems in the BBB, for example insulin and transferrin coupling of a nontransportable drug (peptide or protein) to an anti-receptor antibody or other receptor-specific molecule, results in a chimeric construct that can undergo receptor-mediated transcytosis (Bickel et al., 2003). Drug carriers such as liposomes and nanoparticles have also been used for brain delivery. Despite these developments, there is still a need to develop noninvasive methods which promote the passage of inherently nonpenetrating drugs through the intact brain blood vessel endothelium (Pardridge, 1994).

Recently small peptide-vectors, derived from natural peptides called protegrins, can be used to enhance brain uptake of doxorubicin and penicillin. The potential of this approach as an effective delivery system for transporting drugs across the blood-brain barrier has been demonstrated in a number of animal models. The results obtained in these studies indicate that the use of peptide vectors can enhance significantly the brain uptake of doxorubicin without opening the tight junctions. The mechanism by which this vectorized doxorubicin crosses into the brain has been shown to be an adsorptive-mediated endocytosis process (Rousselle et al., 2001).
To assess the broad potential of this approach, we have coupled dalargin with SynB vectors and measured its brain uptake and pharmacological effect. Dalargin is a hexapeptide analog of Leu-enkephalin containing D-Ala in the second position and an additional C-terminal arginine. These modifications modulate the stability of dalargin in the blood stream and brain, while at the same time modifying to some extent its receptor selectivity. While the intracerebroventricular injection of this peptide has been shown to induce analgesic action, its systemic administration shows no activity in central analgesic mechanisms. The reason for this is because dalargin is known not to cross the BBB. In this study that SynB vectors improve the delivery of dalargin into the brain and that this enhancement in uptake is accompanied by a significant increase in its pharmacological potency in an animal model of nociception. These results support the usefulness of peptide-mediated strategies for improving the availability and efficacy of CNS drugs (Kalenikova et al., 2003).

The endogenous µ-opioid receptor agonist, endomorphin-1 (EM-1), cannot be delivered into the central nervous system (CNS) in sufficient quantity to elicit analgesia when given systemically because it is severely restricted by the blood-brain barrier (BBB). To improve the physicochemical characteristics of EM-1 and subsequently achieve greater BBB permeation, we synthesized a series of EM-1 analogues by combining successful chemical modifications including N-terminal cationization, C-terminal chloro-halogenation and unnatural amino acid (D-Ala, Sar and D-Pro-Gly) substitutions in position 2.

Introduction of D-Ala as well as D-Pro-Gly, but not Sar, in place of L-Pro2, also increased the overall lipophilicity to some extent. Among the peptides tested, intracerebroventricular injection of guanidino-[D-Ala2, p-Cl-Phe4] EM-1 showed the strongest analgesia, being three times more potent than the parent peptide. We also found that in comparison with EM-1, the four D-Ala containing tetrapeptides and the chloro-halogenated D-Pro-Gly containing pentapeptide elicited significant and prolonged central-mediated analgesia upon subcutaneous administration, indicating that more peptides reached the CNS eliciting greater analgesic effect.

Opioid peptides have been designed for the treatment of pain, and the mediation of opioid analgesia has long been thought to occur exclusively within the central nervous system (CNS). However, the inability of peptides to readily penetrate the
blood-brain barrier (BBB) to gain access to the brain and spinal cord and their biological instability have hindered their development for clinical use. It is now well established that the BBB is not only a regulatory interface between the CNS and peripheral circulation, but also a transport and metabolic barrier (Brownson and Davis, 2006). The extent to which peptides transit through the BBB into the CNS is affected by many factors including molecular weight, hydrogen bonding potential, lipophilicity, enzymatic stability (Begley, 1996) and affinity for efflux and carrier mechanisms. Thus, appropriate structural modifications of peptides may modify their physicochemical characteristics and thus, BBB permeation and pharmacological properties following peripheral administration.

Numerous strategies have been developed for enhancing peptide delivery to the CNS. With various modifications that were successful, it may be a good idea to combine them. For this purpose, herein we have taken several approaches that mainly fall into three groups (Temsamani et al., 2000):

1. **Cationization:** There is accumulating evidence that cationization can improve pharmacological parameters of peptides such as membrane permeability and proteolytic stability. A study that cationization of EM-2 by guanidino-addition resulted in a significant increase in metabolic stability, BBB permeability and analgesic profile (Hau et al., 2002). Almost at the same time (Hau et al., 2002; Ogawa et al., 2002) found that N-terminal amidination of several dermorphin tetrapeptide analogues led to compounds with strong and long-lasting activity after oral administration (Ogawa et al., 2002).

2. **Chloro-halogenation at the para position of Phe:** Addition of halogens to enkephalin analogues may enhance overall lipophilicity of the compound resulting in greater BBB permeability. Thus, to ensure the necessary lipophilicity for passive transport of the cationized EM-1 analogues through the BBB, structural modification was also made at the C-terminal.

3. **Replacement of L-Pro in position 2 with unnatural amino acids (D-Ala and Sar) or dipeptide fragment (D-Pro-Gly):** This is mainly for stability enhancement purpose. It was found that incorporation of D-amino acid in position 2 of the peptide sequence significantly increased their stability (Dooley et al., 1994).
In an effort to make EM-1 overcome the problems of enzymatic degradation and inability to cross the BBB into the brain, thus being able to produce analgesia after systemic administration, they had synthesized a series of EM-1 analogues by combined chemical modifications, and determined their opioid receptor affinity and selectivity as well as lipophilicity and stability. EM-1 and EM-2 are two highly selective μ-opioid receptor agonists. However, with relatively rapid degradation and limited delivery to the CNS, it is unlikely that these neuropeptides could be used for the clinical treatment of pain. In regard to stability, a promising solution is to develop stable analogues. The unique approaches undertaken here relied upon utilizing N-terminal cationization by guanylation and unnatural amino acid substitutions in position 2. In addition to the necessity for enzymic stability, it is widely accepted that peptides must cross the BBB to reach the CNS in an amount sufficient to act on appropriate receptors to exert the desired pharmacological effects. Passage of opioids through the BBB to gain access to the CNS by passive diffusion appeared to correlate with their lipid solubility. The more lipophilic a peptide is, the more likely it will interact with the cell membrane which, in fact, is the first step for the transcellular pathway (Habgood et al., 2000).

Meanwhile, by comparing the D values of all analogues, it was concluded that the introduction of D-Ala as well as D-Pro-Gly, in place of L-Pro2, also contributed to the enhancement of the overall lipophilicity, which may be due to the differences in hydrophobicity of each amino acid. On the other hand, entry into the brain is a complex phenomenon which depends on multiple factors. In order to improve bioavailability, the effects of lipophilicity on membrane permeability have to be balanced with first-pass metabolism, for the peptidases within the blood and brain can rapidly degrade most peptides, including naturally occurring neuropeptides. Therefore the stability appears to be a deciding factor for the potency of peptide drugs after systemic administration. The four D-Ala containing tetrapeptides as well as the chloro-halogenated D-Pro-Gly containing pentapeptide produced potent and prolonged analgesia upon subcutaneous administration through a central mechanism. Overall, the present study demonstrated that the modifications used in this research are successful for enhanced EM-1 delivery to the brain, and may serve a role in the
development of novel analogues of EM-1 with increased therapeutic potential (Brownless and Williams, 1993).

Novelty seeking has been described as behavioural activation associated with exploration of novel environmental stimuli and pursuit of and approach to potential rewards. Spontaneous exploratory behaviour in a novel environment has been shown to depend on the integrity of the mesolimbic dopaminergic projections. Degeneration of the mesocorticolimbic dopaminergic system, as well as the nigrostriatal system, has been shown to occur in PD (Jellinger, 1991). In general, suggested that it is the reduced level of mesolimbic dopamine activity in the left hemisphere that is responsible for the lower NS in our PD patients. The lack of correlation between NS and the severity of motor symptoms (which is known to reflect the degree of dopamine loss in the striatum), supports the suggestion that it is the reduction of dopamine in the mesolimbic branch of ascending dopamine projections, rather than in the nigrostriatal pathway, that is responsible for reduced NS. Studies support the suggestion that asymmetric pattern of DA activity is associated with NS (novelty seeking) and HA (harm avoidance). NS, HA was significantly correlated with the severity of bradykinesia, which is known to reflect the degree of dopamine loss in the striatum. Thus, we believe that both depression and HA are related to initial involvement of the right hemisphere in PD, but these two behavioural symptoms reflect different pathophysiological processes. Whereas depression is most likely related to loss of serotonergic activity, possibly in the right hemisphere, HA is related to dopaminergic loss in the right hemisphere. He suggesting that in PD, HA is associated with greater loss of DA in the right hemisphere (Eidelberg et al., 1995).

Analogs of somatostatin are being investigated clinically for the treatment of various malignancies, including brain tumors. In general, studied the ability of three therapeutically promising radioactively labeled somatostatin octapeptide analogs, RC-160, RC-121, and RC-161, to cross the blood-brain barrier (BBB) after peripheral or central injection. The entry rates were different for each compound. By contrast, entry across the intact BBB increased 220 times when RC-160 was given in a serum-free perfusate. This suggests that some serum-related factor, probably the previously described protein binding or an aggregation-promoting factor, is the main determinant in limiting the blood-to-brain passage of somatostatin analogs. Entry into the brain
was not inhibited by the addition of unlabeled analog to the perfusate, showing that passage was probably by diffusion across the membranes that comprise the BBB rather than by saturable transport. By contrast, a saturable system was found to transport peptide out of the central nervous system (CNS). The clearance from the CNS of RC-160 and RC-121, but not RC-161, was faster than could be accounted for by reabsorption of cerebrospinal fluid (Banks et al., 1990).

Carnosine (alanyl-L-histidine) and the related dipeptides anserine (alanyl-N\textsubscript{1}-methyl-L-histidine) presence in the central nervous system (CNS). A protective action of carnosine related dipeptides was also demonstrated on the toxic effects of a truncated form of the neurotoxic amyloid peptide. These properties are of particular interest considering the localization of carnosine-related dipeptides in mature glial elements of the central nervous system. Glial cells play a variety of roles in the nervous system providing structural and trophic support to neurons, and participating with endothelial cells in the formation of the blood brain barrier. Thus, it is likely that these cells are able to produce and release substances such as the carnosine-related dipeptides with the potential role of natural protective agents to prevent several types of brain damage (Preston et al., 1996).

The rate of oxidation of dipeptides by Mn(III) was compared with that of oxidation of amino-acids, Val, Ala, and Gly and it was found that the rate of oxidation of dipeptide was slower than free amino acids. The change is due to the increased difference between the functional groups and consequently weaker electrostatic effects. Hence, the oxidation of dipeptides is expected to be slower than the monomers. Further, an apparent correlation was noted between the rate of oxidation and the hydrophobicity of these sequences, where increased hydrophobicity results in increased rate of oxidation. The order of oxidation of dipeptides was found to Val–Gly > Ala–Gly > Gly–Gly, which is in well agreement with their hydrophobicity (Urry et al., 1993).

New triorganotin derivatives of dipeptides with general formulae R\textsubscript{3}Sn(HL), where R = Me and/or n-Bu and/or Ph and HL is the monoanion of glycylglycine, glycylvaline, glycyleucine, glycylyryptophane and glyclytyrosine have been synthesized and characterized on the basis of infrared, multinuclear NMR and 119Sn Mossbauer spectroscopic studies. All the newly synthesized compounds were
examined for their in vivo anti-inflammatory activity (using the carrageenan-induced paw edema bioassay in rats), acute toxicity (LD$_{50}$) and cardiovascular activity. These compounds were also screened for their in vitro antimicrobial activity against *Staphylococcus aureus* Mau (29/58) and (78/71), *Bacillus subtilis* (18/64), *Escherichia coli* (326/71), *Candida albicans* (Pn-10), *Microsporum gypseum* and *Euglena gracillis*. The results revealed that triphenyltin derivatives exhibited anti-inflammatory activity comparable to that of phenylbutazone with high safety margin (LD$_{50}$ > 500 mg kg$^{-1}$). Further Ph$_3$ Sn (Gly- Val) displays a potent cardiovascular activity. Moreover, most of the compounds displayed appreciable antibacterial activities when compared with ampicillin and norfloxacin. Compounds Ph$_3$Sn(Gly-Gly) and Ph$_3$Sn(Gly- Val) are the most distinctive derivatives identified in the present study because of their promising in vivo anti-inflammatory activity and in vitro antibacterial activity against gram-positive and -negative bacteria (Nath and Pokharia, 2005).

Research around dopamine agonists, they have found a promising compound in S PD148903 that represents a new type of prodrug, which in the rat is bioactivated to the catecholamine S-5,6-diOH-DPAT, known to display mixed dopamine D1/D2 receptor agonist properties just like apomorphine. This prodrug has an enone structure which by an oxidative bioactivation mechanism is converted to the corresponding catechol and is delivered enantioselectively into the CNS. This novel concept has the potential to revolutionize the treatment of Parkinson's disease by competing with L-DOPA, the current treatment of choice (Venhuis and Wikstrom, 2002).
A novel type of orally active prodrug of a potent dopaminergic catecholamine was discovered. Pharmacological evaluation of S-PD148903 in striatal microdialysis showed a rapid onset of action after oral administration and a long duration of action. The striatal presence of a catecholamine was observed only after administration of S-PD148903, indicating an enantioselective bioactivation of S-PD148903 or an enantioselective delivery of the catecholamine. Since we previously reported some close analogues of PD148903 that also induced dopaminergic behavior in vivo, bioactivation to a hydroxylated aminotetralin may be a general feature of this type of compounds. Since SPD148903 is effective in a rat model of Parkinson's disease, S-PD148903 or one of its analogues could be of use in the treatment of Parkinson's disease (Venhuis et al., 2000).

A series of 2-aryldopamine analogues were synthesized and evaluated by David L. Ladd and coworkers for their effects on D₁ and D₂ dopamine receptors. The 2-phenyldopamine and 6-phenylbenzazepine analogues exhibited weak binding to both D₁ and D₂ receptors. The 9-(aminomethyl) fluorenes also exhibited weak D₂ binding however. 2, 5, 6-trihydroxy-9H-fluorene-9-methanmine exhibited D₁ binding comparable to apomorphine. The binding activity has been correlated with the calculated torsion angle of the biphenyl portion of these molecules. Good D₁ dopamine binding occurs when the aromatic rings approach coplanarity: poor binding occurs when the aromatic rings are orthogonal (Ladd et al., 1986).

2 -Phenyldopamine

6 -Phenylbenzazepine
Nicholas J. Bach and coworkers proposed and comparisons with apomorphine, that the rigid pyrroleethylamine moiety of the ergolines is the portion of the molecule responsible for dopamine agonist activity. In support of this hypothesis, bicyclic and tricyclic ergoline have been synthesized. In addition, some pyrazole isosters of these rigid pyrroleethylamines have been made. All of the classes show dopaminergic activity in prolactin inhibition and in lesioned rat turning assays. The most potent drugs, the linear tricyclic pyrazoles are comparable in potency with the highly active ergoline pergolide (Nicholas et al., 1980).

\[ \text{Ergoline} \quad \text{Pyrazole isoster of pyrroleethylamine} \]

\[ \text{Tricyclic pyrazole} \]

Drukarch B and Muiswinkel FL developed an effective causal therapy should be focused on preventing or at least retarding the neurodegenerative process underlying the disease. At the cellular level, PD is characterized by degeneration of neuro melanin containing dopaminergic neurons in the substantia nigra. Neuromelanin formation is the outcome of a process generally known as DA autooxidation, a chain of oxidation reactions in which highly neurotoxic DA quinines are produced. The level of these DA quinines, as estimated by the occurrence of their cysteinyll conjugates, was reported to be increased in the parkinsonian substantia nigra. Hence, stimulation of pathways implicated in the detoxication of DA quinines
in the brain may provide neuroprotection in PD. Besides their in activation through non-enzymatic anti oxidants such as ascorbic acid & glutathione, DA quinines are efficiently inactivated enzymatically by NAD (P) H: quinine oxidoreductase (NQO) & glutathione transferase (S), both of which are expressed in human substantia nigra. The activity of these enzymes, which belong to the group of phase-II biotransformation enzymes, can be upregulated by a large variety of compounds. These compounds include dithiolethiones, phenolic antioxidants, & isothiocyanates, have been shown to be active both invivo & invitro. Thus considering the role of phase-II biotransformation enzymes, in particular NQO & glutathione transferase (S), in the detoxication of DA quinines. They proposed that phase-II enzyme inducers warrant evaluation on their neuroprotective potential in PD (Drukarch and Van, 2000).

Recent advances in molecular biology have established the existence of two families of dopamine receptors: a D_{2}-group comprising D_{2}, D_{3}, and D_{4} receptors and a D_{1}-group incorporating D_{1} and D_{3} receptors. Dopamine (DA) is a major neurotransmitter in the central nervous system. Receptors for this catecholamine are of considerable interest, as they are the principal target for drugs employed in the treatment of neuropsychiatric disorders, such as schizophrenia, drug dependence, and Parkinson's disease. For that reason, dopaminergic ligands have attracted considerable attention (Sit, 2000).

DA receptor agonist activities can be found in several classes of compounds, including 2-phenylethylamines, aporphines, 2-aminotetralins, naphthoxazines, and ergoline derivatives. Dopamine, and most of the known DA receptor agonists, binds with a higher affinity to the DA D_{3} than to the DA D_{2} receptor. Due to the close homology between the DA D_{2} and D_{3} receptors, especially in the TM domains (~80%), it is difficult to predict DA D_{2} versus DA D_{3} receptor selectivity based on receptor models (Malmberg et al., 1994), the observed DA D_{3} selectivity may not be due to a single specific interaction but rather, to a small difference in conformation between the D_{3} and D_{2} receptors.

McDermed et al., elegantly rationalized the hetero chirality of the potent DA receptor agonists by suggesting a model in which different faces of the compound would interact with a putative three-point pharmacophore. An attractive feature of
this model is that it allows a superposition of the pharmacophoric elements of several DA receptor agonists, such as the nitrogens, nitrogen lone pairs, oxygens, and aromatic rings. In addition, the presence of two lipophilic sites which bind the N-alkyl groups has been postulated (Wikstrom et al., 1985) that with a series of octahydrobenzo quinolines, using a pharmacological in vivo model measuring DA D₂ activity, that one of the N-alkyl binding sites can only tolerate N-substituents equal to an n-propyl. Seiler and Markstein (Seiler et al., 2002) conclude that this space-limited accessory binding site, which they call the "small N-alkyl binding site", exists in both main groups of the DA receptors.

Durk Dijkstra and coworkers describes the synthesis and in vitro pharmacology of a novel series of dopaminergic agents in which the classical phenylethylamine pharmacophore is replaced by a thienylethylamine moiety. In general, the novel compounds showed a moderate affinity for the dopamine (DA) D₂ and D₃ receptors. When the thienylethylamine moiety is fixed in a rigid system, the affinity for the DA receptor is significantly increased (Dijkstra et al., 2002).

![Thienylethylamine](image)

Victor Shashoua is studying the receptor that transports cis-docosahexaenoic acid or DHA a naturally occurring fatty acid that forms a significant proportion of the brain’s grey matter across the blood brain barrier. Dr Shashoua was using this receptor to sneak dopamine into the brain. Attempts to boost the level of dopamine in the brain in order to treat Parkinson’s diseases have often been thwarted by the blood brain barrier. But Dr. Shashoua has been able to increase the brain's uptake of dopamine almost eightfold by attaching it to DHA, and getting the receptor to carry it
across. He hoped his DHA - dopamine combination, known as "Doprexin", may make a drug that combats Tardive dyskinesia (Alavijeh et al., 2005).

The dopamine transporter (DAT) normally functions to allow rapid uptake of dopamine (DA) and therefore is important in regulating DA actions. DAT binding decreases with age and is additionally decreased due to neuronal loss in PD. The DAT may also play an important role in neuronal development, appearing first at gestational day (GD) 14. In DAT knockout mice, D1 and D2 DA receptors and preproenkephalin A mRNA levels were decreased from GD 14 on, while dynorphin mRNA levels were increased from GD 17 on thus, changes in DAT number and DA levels may affect neuronal development early on, and may play a role in the etiology of PD later in life (Parker et al., 2001).