1. INTRODUCTION

Human brain the most complex organ of vertebrates is composed of billions of neurons which are the building blocks of the central nervous system (CNS). Normally, once the neurons get damaged or die they can't be repaired or replaced, leading to development of a condition called neurodegeneration. Neurodegenerative disease is an umbrella term used for a range of conditions which principally affect the neurons in the brain. Examples of neurodegenerative diseases include Alzheimer's disease (AD), Huntington's disease, Parkinson's disease, etc [1]. In the present study, we are focusing mainly on the AD.

AD is a progressive neurodegenerative disorder of the brain causing deteriorating cognitive functions in elders. Alois Alzheimer, a German psychiatrist recognized the disease for the first time, so the disease was named after him. In 1906, Dr. Alzheimer observed changes in the brain tissue of a woman who had died because of unusual mental illness. The symptoms included memory loss, language problems and unexpected behaviour. After death, he examined her brain and found many abnormal clumps (now called amyloid plaques) and tangled bundles of fibres (now called neurofibrillary tangles, or phosphorylated tau, *p*-tau) which are still considered as the main pathological features of the AD. An estimated 5.3 million Americans of all ages were suffering with AD in 2015. By 2025, this count is expected to reach 7.1 million, a 40 % rise from the currently affected population [2].

The early characteristic symptoms of AD involve difficulty in recalling recent events called short term memory loss. With the progression of the disease, other symptoms may develop which include disorientation, mood and behavioural swings, loss of motivation and not managing one's self care. Over a time period, various body functions are lost which ultimately lead to death. However, the speed of progression of the disease may vary among different populations. Based on the progression of the disease and appearance of various symptoms, AD is categorized in four different stages: (i) early, (ii) mid, (iii) moderate and (iv) late.

1.1. Pathophysiology

Pathologically, AD is characterized by deposition of amyloid-beta (Aβ) plaques extracellularly and neurofibrillary tangles (NFTs) (phosphorylated tau, *p*-tau) intracellularly, resulting in shrinkage of the brain and loss of cholinergic neurons in the hippocampal and basal

forebrain regions of the brain [3, 4]. Loss of cholinergic neurons is a characteristic feature of the disease which is believed to produce cognitive impairment. Aβ and *p*-tau appear in normal brains too but are found to be very less in quantum. Development of AD is associated with early appearance of amyloid deposits with no noticeable symptoms for many years. It has been well recognized now that the altered processing of amyloid protein from its precursor, amyloid precursor protein (APP), is the key event in AD pathogenesis [5]. Certain evidences, gathered particularly from the genetic examination of several familial AD patients, indicate mutation of the APP gene and of some other genes that regulate amyloid protein processing. The APP gene located in chromosome 21, is found duplicated in Down's syndrome wherein early AD-like dementia and overexpression of APP is observed [6].

According to the amyloid hypothesis, the pathological marker of AD, i.e. amyloid Aβ peptide is predominantly formed in two forms i.e. $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides consisting 40 or 42 residues. In normal physiological conditions, $A\beta_{1-40}$ is generated in small amounts whereas overexpression of $A\beta_{1-42}$ is observed in pathological conditions and due to genetic mutations. Both the fragments have a propensity to aggregate to form amyloid plaques. However, $A\beta_{1-42}$ has a greater tendency to do so which makes it the major culprit in AD pathogenesis. APP, a 770 amino acid long membrane bound protein, is naturally expressed in many cells including CNS neurons. The proteases which cut down the Aβ protein sequence are known as secretases. The α-secretase produces a large extracellular domain as soluble APP. Aβ fragments are formed through cleavage of the protein at two different points by β- and γ-secretases including one in the intramembrane domain of APP. These secretases lack precision and cut APP in an abnormal manner generating different lengths of Aβ fragments including $A\beta_{1-40}$ and $A\beta_{1-42}$. Mutation of the APP gene affects the preferred cleavage points and favor formation of $A\beta_{1-42}$ fragments. Other unrelated presenilin genes form part of the γ-secretases. Mutation of the presenilin genes further increases activity of the γ-secretases. Such AD imparting mutations favor the formation of $A\beta_{1-42}$ fragments which can be identified in plasma and serve as biomarkers in AD. ApoE4, a gene for the lipid transport protein was also observed to be mutated in AD facilitating Aβ aggregation [7].

Another key pathological biomarker of AD is *p*-tau. NFTs are composed of tau proteins. Tau is a normal component of neurons, coupled with intracellular microtubules. In AD, an abnormal phosphorylation of tau proteins occurs, which get deposited intracellularly as

characteristic paired helical filaments which can be viewed microscopically. In neurodegenerative conditions, these filaments aggregate in the form of intracellular neurofibrilllary tangles. Tau phosphorylation is also enhanced by Aβ plaques [8].

The exact mechanism of neurodegeneration caused by Aβ aggregate is still unknown. Some studies have revealed that the aggregated Aβ peptides mediate neurotoxicity due to their ability to increase oxidative stress through spontaneous production of reactive oxygen species (ROS) and free radicals [9]. Oxidative and nitrative stress results in the formation of ROS and reactive nitrogen species (RNS) inducing oxidative injury due to protein oxidation, which ultimately leads to the cellular dysfunction and death. Moreover, Aβ aggregates are known to impart neurodegeneration through apoptosis by disturbing the cell's calcium ion homeostasis. Apoptosis and oxidative stress are directly linked physiological phenomena which are involved in various pathological conditions including AD. Apoptotic cell death comprises of a sequence of events causing activation of caspase cascade which causes fragmentation of the cellular proteins and DNA, leading ultimately to disintegration of the cell [10]. It is now well established that massive neuronal and glial cell death is a common feature in the brains of AD patients because of apoptosis [11].

Additionally, alteration of different neurotransmitters has been observed in AD brains, but a relatively selective loss of cholinergic neurons in the hippocampal and basal forebrain regions is characteristic [3, 4]. These findings led to the development of a pharmacological approach to restore the cholinergic function to treat AD with the use of cholinesterase inhibitors. Levels of acetylcholine, acetylcholinesterase, choline acetyl transferase and choline transporters are significantly decreased in AD which are generally not observed in other pathological conditions such as depression or schizophrenia. Hippocampal region having acetylcholine (ACh) as the major neurotransmitter is considered as the memory origin centre of the brain, therefore degradation of ACh by acetylcholinesterase (AChE) enzyme results in loss of hippocampal cholinergic neurons which leads to memory impairment and ultimately neurodegeneration [12].

Another hypothesis points to a correlation between the dysregulated biometal homeostasis and AD. It has been observed that AD patients have elevated serum level and reduced cortex level of copper along with extracellular Aβ plaques containing copper, iron and zinc. Such biometals are considered as good Aβ plaque aggregators and tau phosphorylation

inducers. Aβ plaques in conjugation with biometals induce ROS generation leading to mitochondrial dysfunction, microglia activation and neuroinflammation. Administration of metal chelators could be effective in such circumstances [13].

1.2. Therapeutics

Current treatment for Alzheimer's includes AChE inhibitors which elicit their effects by increased functioning of the existing cholinergic neurons, improved general cognitive functions and reduced behavioural disturbances [3, 4]. However, butyrylcholinesterase (BuChE) is also a key degrading enzyme for ACh during the late phase of the disease. Thus, AChE and BuChE together terminate the action of ACh. Thus, cholinesterase (ChE) inhibitors are considered as potential agents for symptomatic treatment of AD [3]. NMDA receptor antagonist memantine is also approved for mild to moderate AD [14].

Apart from the available therapies, some novel strategies have also emerged. The main focus in this regard is the development of therapeutic agents targeting the Aβ and tau protein. Followings are various therapeutic drug targets in AD:

1.2.1. Anti-amyloid approach

Anti-amyloid agents target various aspects of APP metabolism as discussed below [15].

1.2.1.1. Targeting amyloid transport

Aβ Oligomers are removed from the brain via several pathways including vascular sequestration of the Aβ oligomers by the soluble form of the low-density lipoprotein receptor-related protein (LRP). With age, LRP expression decreases, resulting in altered efflux of Aβ oligomers in causing their prolonged stay in the brain. It has been reported that antibodies against LPR decrease Aβ oligomers' clearance from the brain suggesting that this can be a potential therapeutic target to treat AD. Aβ oligomers also bind to the 'receptor for advanced glycation end' products (RAGE-multiligand receptor) with high affinity at the blood brain barrier (BBB) that helps them to enter into the CNS, resulting in altered CNS entry, inflammation and neuronal cell death [16].

1.2.1.2. Alteration of secretase enzymes

The activity of α-secretase is controlled by the cell surface receptors and by activation of different signaling cascades like protein kinase C (PKC). Bryostatin 1, an anti-cancer agent with potent PKC stimulant activity was investigated for its use in AD and is currently under phase II clinical trials. Another APP cleaving enzyme β-secretase is also a promising target in AD [17]. GRL-8234 a potent inhibitor of β-secretase has shown beneficial effects in preclinical studies [18].

1.2.1.3. Targeting amyloid aggregation

Tramiprosate, a glycosaminoglycan which effectively binds to the monomeric Aβ and attenuates its oligomerization and aggregation, is under phase II clinical trials. ELND005 (Scyllo-Inositol) has shown promising results in transgenic mice by decreasing the level of insoluble Aβ oligomers. It is currently under phase II clinical trials. Colostrinin has the ability to attenuate the Aβ peptide aggregation and makes the pre-formed fibrils soluble [19].

1.2.1.4. Targeting amyloid clearance

In AD, levels of Aβ degrading enzymes significantly decrease enhancing the process of Aβ aggregation. Reports suggest that the plasminogen activator inhibitor 1 abrogates brain and plasma Aβ oligomer levels in transgenic animals. The peptide hormone somatostatin facilitates Aβ clearance via neprilysin activation [20].

1.2.1.5. Amyloid based vaccine therapy

Amyloid based immunotherapy deals with vaccination of an individual with Aβ oligomers, causing an immune response to attenuate Aβ aggregation and facilitating their clearance. Elan and Wyeth in 2001 initiated the first clinical trial using active immunization of synthetic aggregated $A\beta_{1-42}$ peptide administered in QS21 adjuvant [15]. The outcomes from this study were encouraging showing generation of anti-Aβ antibodies, declining cerebrospinal levels of tau and slowing down of memory impairment [21].

1.2.2. Targeting tau protein

Tau proteins are normally synthesized by neuronal cells to stabilize the microtubules for appropriate morphology, functioning and growth of neurons. So, targeting tau proteins could be an effective therapeutic intervention [8].

1.2.2.1. Inhibition of tau phosphorylation

Amongst the primary enzymes involved in the tau phosphorylation, glycogen synthase kinase 3 (GSK-3) is considered as the major therapeutic target in AD. It has been reported that lithium and valproate having GSK-3 inhibitory activities can attenuate tau pathogenesis. Tideglusib (NP0311112), an irreversible inhibitor of GSK-3 has recently completed phase IIb clinical trials (NCT01350362) [22].

1.2.2.2. Targeting microtubule stabilization

Paclitaxel, a microtubule stabilizer, improves axonal transport, microtubule density and motor function in experiment models of AD. Epothilone D, another microtubule stabilizing agent significantly attenuated microtubule pathogenesis. Neuropeptides like NAP (NAPVSIPQ) and SAL (SALLRSIPA) facilitate microtubule stabilization [23].

1.2.2.3. Attenuating tau oligomerization

Drugs like astemizole and lansoprazole have shown a very strong affinity towards the tau protein. These drugs indirectly attenuate tau-tau interaction. Methylene blue (methylthionium chloride) has the ability to interfere with tau interactions. It also has significant Aβ aggregation and AChE inhibitory effects leading to improved electron transport, reduced oxidative stress and attenuated mitochondrial damage [24].

1.2.2.4. Enhancing tau degradation

It has been documented that heat shock protein 90 (Hsp 90), a chaperon is involved in folding of denatured proteins. Thus, Hsp 90 plays a vital role in attenuating tau degradation. Curcumin has the ability to inhibit Hsp 90 along with other significant effects. Reports have shown that curcumin treatment decreased the tau pathogenesis in transgenic animals by suppressing tangle formation along with clearance of already formed tangles [25].

1.2.2.5. Tau based vaccination therapy

Tau immunotherapy is another approach to facilitate immunological clearance of tau tangles. Recent active immunization studies have given an opportunity to abrogate tau pathogenesis by activation of immune system. Passive immunotherapy was found to be effective in JNPL3 and P301S tauopathy models, however the exact mechanism behind them remains unclear [26].

1.2.3. Targeting intracellular signaling cascade

Aβ peptides activate different intracellular pathways. Therefore, interruption of these signaling cascades could be effective in AD. Reports state that phaosphodiesterase (PDE) inhibitors provide significant improvement in experimental models of AD. It has been shown previously that rolipram, a PDE-4 inhibitor, has efficiently reversed memory and cognitive alterations in Aβ treated mice. Sildenafil, a PDE-5 inhibitor also produced similar results. Recently discovered cilostazol inhibits PDE-3 activity showing beneficial effects in transgenic animal models by attenuating Aβ aggregation and tau phosphorylation. Some other PDE inhibitors, which are in preclinical development stages include AVE-8113, BCA-909 and THPP-1. Inhibition of phospholipase A2 also protects experimental animals from cognitive deficits by attenuating the levels of tau protein. Recently investigated phospholipase A2 inhibitor, rilapladib, is currently under phase II clinical trials (NCT01428453) [27].

1.2.4. Modulation of neurotransmitter levels

1.2.4.1. Cholinesterase inhibitors (ChEIs)

Currently available anti-AD treatment includes four US FDA approved ChEIs namely tacrine, donepezil, rivastigmine and galantamine which enhance brain ACh levels by attenuating ChE activity. Since the last couple of years, memogain (GLN-1062), a benzoyl ester prodrug of galantamine is available as intranasal formulation. Another compound, huperzine A, a natural alkaloid isolated from the Chinese moss shrub (*Huperzia serrata*), has also shown significant neuroprotection. Reports state that muscarinic 1 (M1) AChR agonists play an essential role in APP processing and ultimately modulating other processes such as tau phosphorylation. It has been demonstrated that removal of M1 AChRs leads to increased formation of Aβ peptides.

AF102B, an M1 AChR partial agonist, efficiently reduced CSF Aβ levels in AD patients. AF150(S) and AF267B have also shown beneficial effects in preclinical studies. ANAVEX 2-73, a mixed M/σ1 AChR agonist, is currently under phase I/IIa clinical trials. Recently, use of a nicotine metabolite, cotinine, has been implicated in memory impairment. It is a positive allosteric modulator of α7 nicotinic AChRs [28].

1.2.4.2. Modulation of GABAergic neurons

In neurodegenerative conditions, initially the cholinergic neurons are affected in the hippocampal region followed by the glutamatergic neurons. Reports suggest that under the conditions where chronic growth factor deprivation occurs, the GABAergic transmission turns from an inhibitory stimulus to an excitatory one. Currently, some GABAergic drugs are being evaluated for their cognitive enhancing potential. SGS742, a $GABA_B$ receptor antagonist has demonstrated promising effects in preclinical and phase I clinical stages and is currently under phase II clinical trials. Etazolate, a $GABA_A$ receptor modulator has also shown neuroprotective potential. It activates α-secretase and inhibits PDE-4 activity [29].

1.2.4.3. NMDA receptor antagonist

Glutamatergic neurotransmission regulates neuronal growth and differentiation, synaptic plasticity, cognition, memory and learning. Memantine an uncompetitive NMDA receptor antagonist, blocks the receptor by occupying it in the open state. Memantine improves spatial learning and memory in experimental models of AD, protects neurons against Aβ insult, attenuates apoptosis, scavenges free radicals and restores synaptic degeneration [14]. Memantine is the only drug approved clinically to treat moderate to severe AD in USA and Europe. Cholinergic neurotransmission plays an important role in the early stages of AD while alterations of glutamatergic system and excitotoxic damage occur late during the progression of the disease. Clinical trials of ADS-8704 (a combination of memantine and donepezil, Admas Pharmaceuticals) is going on in moderate to severe AD (NCT00866060). It is currently under phase III clinical trials [30].

1.2.4.4. Modulation of serotonin receptor

Areas of the brain associated with learning and memory functions have significant expression of the serotonin receptors including 5-HT_{1A}, 5-HT_4, 5-HT_6 and 5-HT_7 receptors. Compounds like monoamine oxidase (MAO) inhibitors and SSRIs are already in use clinically in AD as monotherapy or along with AChEIs because of their cognitive enhancing abilities. Novel ligands possessing agonist or antagonist activities at different 5-HT receptors are currently available. Lecozotan, a 5-HT_{1A} antagonist is under phase II clinical trials (NCT00151333) [31]. Several other compounds like PRX-03140, velusetrag, TD-8954, RQ-00000009, SUVN-D1003019 and SUVN-1004028 with 5-HT_4 agonist activity have demonstrated significant improvement in the altered cognition and amyloid processing in preclinical studies. A novel 5-HT_6 agonist, SB-742457, has shown promising results in phase II clinical trials as monotherapy as well as in combination therapy with donepezil. Moreover, 5-HT_6 antagonists like RO-4368554, SB-258585 and SB-399885 have also demonstrated cognition enhancing ability in preclinical studies [32].

1.2.4.5. Histaminergic modulators

Histamine H_3 receptors are predominantly present in those regions of brain which play a vital role in cognitive functions and sleep-wake regulation. Activation of this receptor attenuates histamine release in the brain. However, its selective antagonism potentiates the release of different neurotransmitters including ACh, GABA, dopamine and noradrenaline. Some novel H_3 antagonists like BF2.649, GSK189254, PF-03654746, ABT-288, MK-0249 and JNJ-17216498 have significantly improved cognitive functions in preclinical studies. ABT-288 was found to be well tolerated and safe in healthy adults, and has recently completed phase II clinical trials (NCT010118875) [33]. GSK239512, a selective H_3 antagonist, has shown excellent safety along with improvement in attention and memory functions in a small trial [34].

1.2.5. Targeting mitochondrial dysfunction

Aβ Peptides inhibit mitochondrial transport channels resulting in reduced activity of complex IV and elevated ROS production. Increased ROS levels further impart mitochondrial dysfunction and ultimately cell death. CoQ10 shows potential neuroprotective effects through suppression of ROS production and stabilization of mitochondrial functions. Idebenone, a water

soluble analog of ubiquinone improves cognitive alterations and attenuates the disease progression. Meththylene blue also serves as an alternate electron carrier through bypassing complex I/III blockage and may impart neuroprotection in AD. Lipoic acid in combination with vitamin C and E has also halted oxidative stress in AD. Lipoic acid and omega-3/fatty acids combination therapy is currently under phase I/II clinical trials (NCT01780974, NCT01058941). Szeto-Schiller peptide (SS-31) is also a novel mitochondrial targeted ROS scavenging therapy [35].

1.2.6. Targeting oxidative stress

ROS affects various key biomolecules like enzymes, membrane lipids, DNA etc which would ultimately lead to cell death. Several natural antioxidants like, vitamins (C, E and carotenoids) and phytochemicals have shown significant neuroprotection in AD. Combination of vitamin E and memantine has recently completed phase III trials, and the results are awaited. Vitamin E in combination with selenium is currently under phase III clinical trials (NCT00040378). Some ubiquitous antioxidants like flavanoids, rutin and carotenoids have also demonstrated promising effects in experimental models of AD. Another potent antioxidant, melatonin is currently under phase II clinical trials (NCT00940589). Recently, a novel melatonin agonist Neu-P11 has been discovered showing memory improvement and neuroprotection [36].

1.2.7. Anti-inflammatory therapy

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to control neuroinflammation during the disease progression as they exert promising effects via various mechanisms apart from their cyclooxygenase (COX) inhibitory effect, like maintaining calcium homeostasis, targeting γ-secretase, Rho-GTPases and peroxisome proliferator-activated receptor (PPAR). Through Rho-GTPases pathway, NSAIDs control various processes associated with AD including axon growth, tau phosphorylation and astrocyte motility. SC-560, a selective COX-1 inhibitor has shown beneficial effects in triple transgenic mice by reducing the inflammation and neuropathology and reversed the cognitive impairments [37].

1.2.8. Multi-target-directed ligands

As discussed earlier, AD is a complex neurodegenerative disorder associated with multifactorial etiology. To face the complexity of the mechanisms involved in AD, compounds with several potential targets have been designed currently which interacted through different mechanisms and provided symptomatic as well as disease modifying outcomes. For example, compounds with AChE and BACE inhibitory activities or AChEI with antioxidant property could be of use in AD [38]. Ladostigil (TV3326) [(N-propargyl-(3R)-aminoindan-5-yl)ethyl methyl carbamate], a multifunctional compound acts as AChEI, hence improves cholinergic neurotransmission, and also possesses MOA-A and B inhibitory effects. It attenuates amyloidogenic APP processing and imparts neuroprotective and neurorestoretive properties and decreases apoptosis. Ladostigil exhibits the neuroprotective effects while rasagiline, an anti-Parkinson's drug with selective MAO-B as well as the ChE inhibitory activities makes it a potential drug candidate to treat Lewy body disease and AD [39].

1. INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is probably a unique monoamine neurotransmitter. The identification of 5-HT as a neurotransmitter involved multidisciplinary efforts by several research groups. Vittorio Erspamer in Rome, for the first time identified the effects of an acetone extract of enterochromaffin cells isolated from gastrointestinal (GI) mucosa. He observed that a compound present in the extract caused constriction of the rat uterus smooth muscle. After several chemical tests, he found that the responsible compound is an indole and was named as enteramine [1]. Later, Maurice Rapport, Arda Green, and Irvine Page established that enteramine contained a unique active component which was named serotonin. Irvine Page was working on the isolation of substances from the blood having vasoconstrictor effects which could be playing a role in hypertension. He identified that upon blood coagulation, a vasoconstrictor substance was released instantaneously. The released material was separated and purified by Maurice Rapport and Arda Green [2]. Rapport then carried out the isolation and purification of serotonin from approximately 900 liters of serum isolated from the beef blood along with its structure identification [3]. The isolated compound was ultimately purified, crystallized and named serotonin in 1948. The compound was named as serotonin because it was isolated from serum ("ser") and induced contractions or increased tone ("tonin") of blood vessels. Based on the classical chemical structure elucidation approach Rapport then proposed that serotonin was "5-hydroxytryptamine, 5-HT". In 1951, Hamlin and Fischer confirmed the structure by the chemical synthesis of serotonin [4]. Later, Erspamer and Asero also synthesized serotonin and established that it was similar to the enteramine which was isolated from the natural source [5]. Commercial availability of synthetic serotonin then created ample opportunities for rapid discovery of several vital physiological functions of serotonin.

The above findings had restricted the role of serotonin only to the peripheral vascular system as a compound that induced constriction of blood vessels and smooth muscles. Later, Betty Twarog prepared acetone extracts of different mammalian tissues including brain and quantified the amount of serotonin in them [6]. Surprisingly, noticeable amount of serotonin was detected in the brains of rats, dogs and rabbits. The discovery of serotonin in the brain was eventually supported by the finding of potent mind-altering effects of lysergic acid diethylamide (LSD) by Dr. Albert Hofmann in 1943 [7]. It was readily identified that the tryptamine portion of

the LSD structure was also the part present in serotonin. In this circumstance, Woolley and Shaw first noted that "the mental disturbances caused by LSD were to be attributed to the interference in the actions of serotonin in the brain". Later, Woolley and Shaw demonstrated through some experiments that LSD had properties similar to serotonin [8]. These findings proposed that alterations in brain functioning could be attributed to bahavioural and psychiatric disorders. This proposal imparted a significant influence on the research on brain and introduced a new era of modern neuropsychopharmacolgy. Rapid investigations of the role of serotonin in behaviour had been initiated which has continued unabated till today. It has now been revealed that serotonin plays a pivotal role in normal brain functions including modulation of hunger, mood states, sex, anxiety, emotion, endocrine effects, sleep, memory and many more [9, 10].

5-Hydroxytryptamine (serotonin, 5-HT) imparts its effects via as many as fourteen different membrane bound receptors. 5-HT and its receptors are predominantly expressed in the central and peripheral nervous systems (CNS/PNS), and also in different nonneuronal tissues like gut, blood and cardiovascular system. 5-HT and its receptors have also been involved in various pathological conditions like depression, obsessive-compulsive and panic disorders, anxiety, eating disorder, schizophrenia, social phobia, hypertension, irritable bowel syndrome (IBS), migraine and vomiting [9].

1.1. Classification of 5-HT receptors

Currently, eighteen genes have been identified which are responsible for expressing fourteen different mammalian 5-HT receptor subtypes. They are classified into seven distinct families. All 5-HT receptors are from the G-protein-coupled receptor (GPCR) superfamily, except 5-HT$_3$ receptor, which is a Cys-loop lignad-gated ion channel. [9]. Further heterogeneity is developed via alternate splicing (e.g. 5-HT$_3$, 5-HT$_4$ and 5-HT$_7$ receptors), RNA editing (5-HT$_{2C}$ receptor) and the formation of homo and heterodimers (5-HT$_4$ and β-adrenoceptor).

1.1.1. 5-HT$_1$ receptor family

The 5-HT$_1$ receptor family consists of 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ receptor subtypes which in humans share 40-63 % overall sequence homology and preferentially couple to G$_{i/o}$ to inhibit adenylate cyclase and reduce the levels of cyclic adenosine monophosphate (cAMP). The 5-HT$_{1E}$ and 5-HT$_{1F}$ receptors are endogenous receptors with

unknown physiological role. However, 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors have been identified functionally in different tissues from various species. The 5-HT$_{1C}$ receptor was then considered as 5-HT$_{2C}$ receptor due to its similar structural, functional and transductional properties with the 5-HT$_2$ receptor family [9, 10].

1.1.1.1. 5-HT$_{1A}$ receptors

5-HT$_{1A}$ receptors are highly expressed in the CNS, mainly in the dendrites, soma, in several axon hillocks of neurons, and in the astrocytes. These receptors are present in all the serotonergic neurons in the form of autoreceptors and in several non-serotonergic neurons as heteroreceptors. 5-HT$_{1A}$ receptor activation induces inhibitory electrophysiological activities in the neurons by reducing neuronal firing rate. 5-HT$_{1A}$ receptors are considered as important therapeutic targets in various neuropsychiatric disorders like anxiety, depression and schizophrenia. Several highly selective compounds have been identified ranging from full agonists to partial agonists, inverse agonists and antagonists. Some atypical antipsychotics used clinically have fractional 5-HT$_{1A}$ agonist or antagonist activity. Buspirone, used in generalized anxiety disorder, is a partial agonist of 5-HT$_{1A}$ receptor. Partial agonists act upon autoreceptors and could become clinically effective anxiolytics. In contrast, in the hippocampus, 5-HT$_{1A}$ receptors develop antidepressant action through promoting neurogenesis and regulating hypothalamic-pituitary-adrenal axis. Apart from this, CNS 5-HT$_{1A}$ receptor activation is also involved in hypothermia, hyperphagia and serotonin syndrome [9]. 5-HT$_{1A}$ receptor knockout mice exhibit increased anxiety and decreased depression like conditions. This may be due to different actions of the receptors in the early development of brain and in expressing emotions in the adult. There are several highly selective 5-HT$_{1A}$ receptor compounds that have been developed. However, some of them share affinity to 5-HT$_7$ (8-OH-DPAT) other 5-HT$_1$ or 5-HT$_2$ receptors. Xaliproden and S-14506 are selective agonists while WAY100635 is a highly selective 5-HT$_{1A}$ receptor neutral antagonist.

1.1.1.2. 5-HT$_{1B}$ receptors

5-HT$_{1B}$ receptors are also widely expressed in CNS in the serotonergic as well as non-serotonergic systems. β-Adrenergic receptor antagonists possess high affinity for 5-HT$_{1B}$ receptors in some species but not in all. 5-HT$_{1B}$ autoreceptors inhibit 5-HT biosynthesis and

release, and increase reuptake through the 5-HT transporters. 5-HT$_{1B}$ heteroreceptors attenuate the release of various neurotransmitters, based on the type of neurons. Systemic application of 5-HT$_{1B}$ agonist induces increased locomotor activity and altered brain reward mechanism, while selective antagonists exhibit precognitive effects. Several 5-HT$_{1B/1D}$ antagonists are effective for antimigraine therapy. 5-HT$_{1B}$ knockout mice showed increased aggression and addiction-like behaviour. These phenotype changes however are attributed to compensatory alterations in the dopaminergic system during the development rather than the reduced 5-HT$_{1B}$ receptor signaling. A number of moderate selective agonists like CP93129 and CP94253, and antagonists like SB224289 are commonly used to study 5-HT$_{1B}$ receptor effects.

1.1.1.3. 5-HT$_{1D}$ receptors

In the brain, 5-HT$_{1D}$ receptors are present in lower concentrations than 5-HT$_{1B}$ receptors, with the highest expression in the raphe nuclei. In addition, 5-HT$_{1D}$ receptors are present in the heart affecting the release of 5-HT. Currently used antimigraine agents have affinity for both 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. It has been suggested that neurogenic inflammation and nociceptive action in trigeminovascular afferents are mediated through 5-HT$_{1D}$ receptors rather than 5-HT$_{1B}$ receptors. Selective 5-HT$_{1D}$ receptor agonist PNU109291, plays an important role in the inhibition of trigeminal nociception and meningeal neurogenic inflammation in guinea pig, supporting the assumption that 5-HT$_{1D}$ receptors are valuable therapeutic targets for migraine and associated headaches [11].

1.1.1.4. 5-HT$_{1e}$ receptors

The lower case letter denotes that the receptor does not have meaningful physiological actions. The receptor was first recognized in radioligand binding studies of human frontal cortex homogenate. The genetic sequencing of 5-HT$_{1e}$ receptor has been done from the human placental library and guinea pig brain genomic DNA but it has not been identified in mouse and rat. There is a lack of highly selective ligands but various typical 5-HT$_{1}$ receptor agonists and antagonists possess modest binding affinity for 5-HT$_{1e}$ receptors [12]. As discussed above, their physiological significance remains unclear.

1.1.1.5. 5-HT$_{1F}$ receptors

The 5-HT$_{1F}$ receptor has been identified in various animals including human, guinea pig, rat and mouse. Similar to other 5-HT$_1$ receptor family members, it inhibits adenylate cyclase via G_i-dependant mechanism. A modest expression of this receptor has been found in the CNS in the serotonergic as well as nonserotonergic neurons, serving as auto and heteroreceptors respectively [9]. Similar to 5-HT$_{1B}$, 5-HT$_{1F}$ receptor is present in trigeminal ganglia and vestibular neurons, having strong affinity towards the triptan drugs which are used in migraine. A less selective agonist can potentially activate multiple 5-HT$_1$ receptors (i.e. 5-HT$_{1B/D/F}$, like triptans) and thus reduces migraine headache through different mechanisms. LY334370, a selective 5-HT$_{1F}$ agonist having ~100 fold greater binding affinity towards 5-HT$_{1F}$ over 5-HT$_{1B}$ receptor, has shown antimigraine activity in animal models through its action on the trigeminal nucleus.

1.1.2. 5-HT$_2$ receptor family

5-HT$_2$ receptor family comprises of three members namely 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors having 46-50% sequence similarity. 5-HT$_2$ receptors couple to $G_{g/11}$ to elevate the inositol phosphate hydrolysis and increase calcium level. 5-HT$_2$ receptors possess high clinical significance due to their complex pharmacological features [9]. 5-HT$_2$ receptors were initially classified by Gaddum and Picarelli in 1957 [13]. They identified two types of tryptamine receptors: M and D receptors which in the current nomenclature are being considered as 5-HT$_3$ and 5-HT$_2$ receptors.

1.1.2.1. 5-HT$_{2A}$ receptors

5-HT$_{2A}$ receptors are abundantly available in the forebrain, mainly in the cortex, pyramidal and interneurons. Apart from CNS, 5-HT$_{2A}$ receptors are also found in various smooth muscles like uterine, bronchial and urinary smooth muscles mediating contractive responses. Moreover, 5-HT$_{2A}$ receptors play an important role in elevated capillary permeability and platelet aggregation [9]. Different patterns of pharmacological activities are displayed by various ligands ranging from full to partial agonists and from neutral antagonists to inverse agonists. Some agonists like 2, 5-dimethoxy-4-iodoamphetamine (DOI) and LSD have greater affinity toeards 5-HT$_{2A}$ receptors, however they also bind to other 5-HT$_2$ receptors. Ketanserin and MDL100907 are well characterized highly selective 5-HT$_{2A}$ receptor antagonists. There are several other

ligands possessing higher binding affinity. 5-HT$_{2A}$ agonists induce psychotomimetic actions (mainly LSD) and thus many antipsychotic drugs act as 5-HT$_{2A}$ receptor antagonists [14].

1.1.2.2. 5-HT$_{2B}$ receptors

There is an abundance of 5-HT$_{2B}$ receptors in kidney, liver, fundus and heart with the minimum presence in discrete subregions of CNS. This characteristic distribution put 5-HT$_{2B}$ receptors aside from other 5-HT$_2$ receptors which have a predominant presence in CNS. The exact role of 5-HT$_{2B}$ receptors has not been fully elucidated yet. However, they are involved in cardiac function, morphogenesis and anxiety. Many drugs possess affinity for all the 5-HT$_2$ receptors. However, a few selective 5-HT$_{2B}$ antagonists like RS127445 have been identified [15].

1.1.2.3. 5-HT$_{2C}$ receptors

5-HT$_{2C}$ receptors are more abundant throughout the CNS but have low levels in the periphery. They are distinctive amongst 5-HT$_2$ receptors due to their editable mRNA transcript, leading to subtle changes in coding sequence, which imparts functional impact on mature receptor protein. 5-HT$_{2C}$ receptor knockout mice exhibit glucose intolerance, mid-life obesity and seizures [16]. The pharmacology of 5-HT$_{2C}$ receptor is identical to 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, showing complex communications with different signal transduction mechanisms. MK212 and RO600175 have shown moderately selective agonist activity while LY53857, ZM170809, ritanserin, mianserin and mesulergine possess nonselective antagonist activity. *m*-CPP mediated anxiogenic response is attributed to 5-HT$_{2C}$ receptor activation, while the selective 5-HT$_{2C}$ antagonists like SB242084 show anxiolytic effects in different experimental models. *m*-CPP and RO600175 administration have shown additional characteristic behavioural responses through 5-HT$_{2C}$ receptor activation which include oral dyskinesia, hypoactivity, increased penile grooming/erections and hypophagia [17]. RS102221 increases food consumption and ultimately weight in animals via its 5-HT$_{2C}$ receptor antagonist activity. 5-HT$_{2C}$ receptor is considered as a useful target in feeding disorders [18].

1.1.3. 5-HT$_3$ receptors

Among all of the 5-HT receptor subtypes, 5-HT$_3$ receptors are the only Cys-loop ligand-gated ion channels. The receptor complex is made up of five subunits 5-HT$_{3A-E}$. The 5-HT$_3$

receptor complex is a nonselective cation channel (mainly permeant to calcium, sodium and potassium ions) mediating fast synaptic neurotransmission. Brain has the highest expression of 5-HT$_3$ receptors which are highly expressed in the brainstem nuclei containing the chemoreceptor trigger zone, namely the nucleus tractus solitaries, area postrema and dorsal motor nucleus of the vagus nerve. 5-HT$_3$ receptors are also found in different regions of forebrain like amygdala, hippocampus and caudate-putamen [19]. There are several 5-HT$_3$ receptor agonists like tropisetron, ondansetron, palonosetron and granisetron that have demonstrated beneficial effects in alleviating nausea and vomiting during radio- and chemotherapy of cancer and also in postoperative vomiting especially observed during abdominal events [19].

1.1.4. 5-HT$_4$ receptors

The 5-HT$_4$ receptor protein also develops from a single gene, like other GPCRs. Its mRNA has the ability to undergo alternate splicing, producing ten isoforms 5-HT$_{4(A-G)}$, 5-HT$_{4(HB)}$, 5-HT$_{4(I)}$ and 5-HT$_{4(N)}$. Except for the 5-HT$_{4D}$ receptor isoform, all 5-HT$_4$ receptor transcripts are present in the brain. 5-HT$_4$ receptors are mainly expressed in the brain, gut and cardiovascular tissues. In the brain, the highest expression is observed in the caudate nucleus, hippocampus, substantia nigra, cortex, globus pallidus, nucleus accumbens and putamen. 5-HT$_4$ receptors play a pivotal role in memory and learning. Several studies indicate that activation of 5-HT$_4$ receptors improve cognitive functions. This effect may be attributed due to their ability to improve acetylcholine release from the cerebral cortex. 5-HT$_4$ receptors are also involved in the metabolism of amyloid precursor protein (APP). They promote sAPPα secretion a neuroprotective peptide, that facilitates neuronal growth and improves memory functions and ameliorates cellular toxicity associated with excessive glutamatergic neurotransmission [20]. 5-HT$_4$ receptor activation also increases anxiety. Therefore, 5-HT$_4$ receptor antagonists exhibit anxiolytic properties. Several drug tools have been identified which may antagonize or activate 5-HT$_4$ receptors. GR113808, SB204070 and RS10235 are high affinity 5-HT$_4$ antagonists while RS67506, ML10302 and BIMU8 are selective agonists [21].

1.1.5. 5-HT$_5$ receptors

5-HT$_5$ receptor family consists of two gene products, 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors. They are the least known 5-HT receptor subtypes. In the rat brain, 5-HT$_{5A}$ receptor protein expression has been identified in hypothalamus, raphe nuclei, locus coeruleus, cerebral cortex, hippocampus, substentia nigra, ventral tegmental area, pons and cerebellum. In the rat brain, mRNA expression of 5-HT$_{5B}$ receptor has been found in entorhinal, hippocampus, olfactory bulb and piriform cortices. A definitive role of 5-HT$_5$ receptors has not yet been identified. 5-HT$_{5A}$ receptor knockout mice demonstrate increased exploratory behaviour in new environments. 5-HT$_{5A}$ receptor also regulates rodent circardian rhythm. SB699551-A shows a 30-fold higher affinity for 5-HT$_{5A}$ receptor over other 5-HT receptor subtypes [10, 22].

1.1.6. 5-HT$_6$ receptors

5-HT$_6$ receptors are coupled to G$_s$ to activate adenylate cyclase. They are strongly and selectively distributed in the CNS. In animal models, 5-HT$_6$ receptor has been identified as an important therapeutic target for cognitive enhancement and possibly in weight loss. A number of selective 5-HT$_6$ receptor agonists have been discovered including 2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine (EMDT), EMD386088 and WAY181187, and several antagonists like SB399885, SB258585 and RO4368554 have also been developed. There are several clinically used antidepressant and antipsychotic drugs having greater binding affinity for 5-HT$_6$ receptor over several other therapeutic targets [23, 24].

1.1.7. 5-HT$_7$ receptors

5-HT$_7$ receptors are also coupled to Gs with wide distribution in the brain. A number of drugs used in depression and psychosis have shown significant affinity for 5-HT$_7$ receptors even at commonly used doses. Several ligands which are traditionally linked with other 5-HT receptors mainly 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_6$ also have the ability to bind to 5-HT$_7$ receptor. 8-OH-DPAT, has 10 fold greater binding affinity for 5-HT$_{1A}$ receptor as compared to 5-HT$_7$. The 5-HT$_7$ receptor has been involved in different behavioural and physiological activities including affective behaviour, vasodilation and circardian rhythm. In the forced swim test, 5-HT$_7$ knockout animals exhibit decreased immobility. These results are supportive to the finding that blockage of 5-HT$_7$ receptor can cause antidepressant action. Some 5-HT$_7$ receptor agonists, including

AS19 and LP12 have been reported. Some selective 5-HT$_7$ receptor antagonists have also been developed including SB258719 and SB269970 [25].

1.2. Therapeutic implications of 5-HT$_{2C}$ receptors

5-HT$_{2C}$ receptors have been implicated in several pathological conditions as discussed below.

1.2.1. Obesity

It is well-known that increasing the brain 5-HT neurotransmission controls the feeding habit by inducing hypophagia (decreased food intake). Before the introduction of serotonergic anti-obesity drugs, the traditionally used pharmacological treatments caused behavioural alterations along with weight loss. Usage of amphetamines in controlling hunger was associated with their undesirable psychological effects with abuse potential. Several other monoaminergic drugs such as phenteramine, diethylpropion and phenylpropanolamine with lower abuse potentials were also used but they induced insomnia, anxiety and irritability [26]. Later, fenfluramine and *D*-fenfluramine were introduced with lack of behavioural disturbances and abuse potential. They cause 5-HT release from the synapse and reduce its reuptake and thereby induce hypophagic effect. However, all forms of fenfluramine induced serious adverse effects like pulmonary hypertension and valvular heart disease. In spite of that, the serotonergic drugs are considered as the most important class of appetite suppressant till date as they regulate appetite without inducing any undesirable behavioural side effects. After the discovery of fenfluramine, the first anti-obesity drug, serotonergic drugs remain as interesting drug candidates in the clinical development [27].

As discussed earlier, fourteen 5-HT receptor subtypes are known [9], among which 5-HT$_{2C}$ receptors are the primary targets in regulating the effects of several 5-HT responsive drugs on appetite expression [28]. The 5-HT neurons are directed towards the hypothalamic region which is considered as an important area for energy regulation. It consists of hypothalamic anorexiogenic neuropeptide melanocortin (MC) system which controls the effects of serotonergic drugs in feeding. Activation of 5-HT$_{2C}$ receptor on the pro-opiomelanocortin (POMC) neurons induce hypophagic response by facilitating the release of the endogenous agonist α-melanocyte-stimulating hormone (α-MSH) and subsequent activation of melanocortin receptors 3 and 4

(MC_4R and MC_5R) on the neurons localized in the paraventricular nuclei (PVN) of hypothalamus. 5-HT_{2C} receptor also inhibits the release of the endogenous antagonist of the MC_4R, i.e. agouti-related peptide (AgRP)/neuropeptide Y (NPY). Thus anorectic POMC/MC pathway is negatively regulated by AgRP/NPY neurons located within the arcuate nucleus [27, 28].

5-HT_{1B} receptor activation releases NPY from AgRP/NPY neurons which abrogate POMC activity to facilitate appetite and energy intake. Majority of the serotonergic compounds activate anorexiogenic POMC neurons and attenuate the activity of orexigenic AgRP/NPY neurons to promote satiety and reduced intake of food. Lorcaserine, a selective 5-HT_{2C} receptor agonist, interacts with POMC but not with AgRP/NPY neurons and thus produces net anorexiogneic effects [28].

Several 5-HT_{2C} agonists have demonstrated reduced food intake in various animal models which has been significantly inhibited by the selective 5-HT_{2C} antagonists. Sibutramine induces satiety effects at least in part by enhancing the level of serotonin through 5-HT_{2C} receptor action. Recent research is focusing on finding of more selective 5-HT_{2C} receptor agonists over other 5-HT_2 receptor subtypes, especially over 5-HT_{2B} receptor, as its agonism leads to increased risk of cardiac valvulopathy and pulmonary hypertension [29]. Arena Pharmaceuticals, in 2004, published a series of 3-benzazepine derivatives as 5-HT_{2C} receptor agonists. Some of them showed significant hypophagic response in normal rats and one among them afterward entered into clinical trials. This lead compound was APD356 (lorcaserin), having 100-fold and 15-fold higher binding affinity for 5-HT_{2C} receptor over 5-HT_{2B} and 5-HT_{2A} receptors respectively [27] and had progressed to advanced clinical studies. Lorcaserine lowered body weight and food intake in overweight/obese patients in phase II and phase III clinical trials with least side effects like headache, dizziness and nausea [30].

1.2.2. Anxiety

Serotonergic system has been implicated in anxiety, but the exact mechanism through which 5-HT system contributes towards the disease has not been fully elucidated till date. Among the 5-HT receptor family, 5-HT_2 subtypes play a predominant role in the pathogenesis of anxiety. Emerging evidences have revealed the role of 5-HT_{2C} receptors in the expression of

specific symptoms of anxiety [31]. As discussed earlier, 5-HT$_{2C}$ receptors are highly expressed in the different regions of CNS. Extensive evidences, revealing anxiogenic effects of 5-HT$_{2C}$ receptor agonists in rodents and humans [32] have identified special brain regions which are involved in the specific behavioural effects of 5-HT$_{2C}$ receptor activation. Anxiety-like behaviours including social avoidance [33] and exaggerated shock-elicited fear etc are believed to be elicited through site specific activation of 5-HT$_{2C}$ receptors in the regions of lateral/basolateral amygdala. The amygdale region of brain is classically implicated in fear and anxiety [34].

A mixed 5-HT$_{2B/2C}$ agonist, *m*-CPP elicits anxiogenic effects in rodents and humans [35] which are significantly blocked by different 5-HT$_{2C}$ receptor antagonists when given systemically or injected directly into the amygdala. These findings further support the critical involvement of 5-HT$_{2C}$ receptors of amygdale region in regulating anxiety-like behaviour [36]. Further, the role of amygdala in anxiety has been supported by the observation that microinjection of the 5-HT$_{2C}$ agonist *m*-CPP or CP809010 into amygdala develops anxiogenic effects which are abolished by 5-HT$_{2C}$ antagonists like SB-242048 [36]. SSRIs, the commonly used antidepressants exert some anxiogenic effects through 5-HT$_{2C}$ receptor activation [33, 35]. Thus, in support of the above findings, 5-HT$_{2C}$ receptor antagonists can be used as effective anxiolytic agents devoid of several side effects commonly associated with benzodiazepines (especially tolearance and dependence) and SSRIs (e.g. agitation, sexual dysfunction, insomnia etc)

1.2.3. Depression

Depression is a leading disorder which affects 10-20% of the world population for at least once in their lifetime. Globally, 350 million people of all ages are estimated to be suffering from depression. The role of monoamine system in pathophysiology of depressive response has been well established. The critical areas of the brain including limbic region which modulate behavioural and emotional functions, are densely innervated by the monoamine neurons. Therefore, the receptors regulating monoamine neurotransmission might be the primary targets for antidepressant drugs. 5-HT$_{2C}$ receptors can modulate the 5-HT, dopamine (DA) and norepinephrine (NE) neurotransmission in the brain. It has been established that 5-HT$_{2C}$ receptors negatively regulate the firing activity of 5-HT and DA neurons in the dorsal raphe

nuclei (DRN) and ventral tegmental area (VTA), respectively. 5-HT$_{2C}$ receptor agonists reduce the 5-HT and DA neuron firing activity [37, 38]. SB242084, a selective 5-HT$_{2C}$ receptor antagonist has also shown increased 5-HT and DA neuron firing on its own to demonstrate antidepressant effects. Moreover, several compounds used clinically as antidepressants are potent 5-HT$_{2C}$ receptor antagonists. *m*-CPP, a 5-HT$_{2C}$ receptor agonist causes depressogenic response in various experimental animal models [39].

The exact mechanism of 5-HT$_{2C}$ mediated attenuation of 5-HT and DA neuron firing has not been fully elucidated. 5-HT$_{2C}$ receptors are coupled to GQ11 and G13 proteins out of which the GQ11 driven signal transduction pathway is relatively well characterized. The pathway involves inositol triphosphate (IP3) mediated increased calcium influx into the neuronal cytoplasm which is subsequently followed by the activation of phospholipase C (PLC) and neuronal excitability [40]. However, the inhibitory effects of 5-HT$_{2C}$ receptors on 5-HT and DA neurons' firing are thought to be mediated, at least in part, via γ-aminobutyric acid (GABA) neurons and GABA receptors. 5-HT$_{2C}$ receptors have been shown to stimulate the firing activity of GABA interneurons in the VTA imparting inhibitory effects on the monoamine neuron firing [41]. Thus, 5-HT$_{2C}$ receptor antagonism can be useful to treat depression, all alone or with SSRIs.

1.2.4. Penile erection

Penile erection is a complex neurovascular process in which nerve, endothelium of blood vessels, sinusoids and smooth muscle cells of the target organ are occupied. Penile erection is mainly caused by a spinal reflux arising from different central and peripheral neuronal and/or hormonal mechanisms [42]. Role of 5-HT$_{2C}$ receptors in penile erection has been well established. *m*-CPP, a 5-HT$_{2C}$ receptor agonist elicits penile erection and excessive grooming in various experimental animal models along with elevated levels of prolactin, oxytocin and corticosterone. The hypothalamic paraventricular nucleus plays an important role in regulation of these behaviours and neuroendocrine effects [43]. Bancila *et al* (1999) [42] demonstrated that the supraspinal serotonergic control of penile erection at the lumbosacral level is strongly attributed to the 5-HT$_{2C}$ receptor activation. RO600175, a selective 5-HT$_{2C}$ receptor agonist also elicits penile erection similar to *m*-CPP. The effects are significantly reversed by SB200646 and SB206553, which act as potent 5-HT$_{2C}$ receptor antagonists for 5-HT$_{2A}$ receptor [42].

1. INTRODUCTION

In the industrialized countries, a marked prevalence of neurodegenerative diseases has been observed with prolongation of lifespan. Human neurodegenerative diseases are associated with the progressive dysfunction and loss of neurons similar to those elicited by particular neurological insults. Research over the past several decades suggests that excitatory amino acids play an important role in the psychological functions like memory and learning. Alteration of the excitatory amino acid system may develop schizophrenia and neuropsychiatric syndromes like dementia and delirium. Overactivation of the excitatory amino acid system causes excitotoxicity. Among the excitatory amino acid system, glutamate is the chief excitatory neurotransmitter of the mammalian central nervous system (CNS). The neuronal excitotoxicity generally cause injury and death of neurons due to prolonged exposure to glutamate and the associated massive ion influx into the cell [1].

A very first toxic effect of glutamate was identified by Lucas and Newhouse who demonstrated degradation of the inner layer of retina in infant mice by subcutaneous injections of glutamate [2]. Later, Olney introduced the term "glutamate excitotoxicity" by describing the intracranial brain lesions in infant and adult mice by subcutaneous injections of glutamate [3]. Shortly thereafter, Olney and Sharpe reproduced the same results in primates, although it required high concentrations of glutamate. In this finding, it was observed that the periventricular areas and hypothalamus regions of the brain were more responsive to systemic glutamate toxicity [4]. An identical neurodegeneration was seen after cerebral ischemia, suggesting that glutamate excitotoxicity played a crucial role in ischemic neuronal cell death. Rothman supported this finding by demonstrating that ischemic insult in hippocampal cell culture was attenuated by a postsynaptic excitatory amino acid (EAA) inhibitor, gamma-*D*-glutamyglycine [5]. Since these findings, glutamate excitotoxicity has been closely associated with ischemic CNS damage and other pathological conditions in brain of related mechanism. Over the past several decades, glutamate excitotoxicity has been found to be associated with different neurodegenerative conditions like Alzheimer's disease (AD), Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) [6].

Glutamatergic neurotransmission is mediated through different glutamate receptor subtypes. Following is a brief introduction about these receptors.

1.1. NMDA receptors

Among all of the glutamate receptors, NMDA receptors (NMDARs) have gained the highest attention. The receptor has been named so because of its binding affinity towards *N*-methyl-*D*-aspartate (NMDA). NMDARs are involved in different neurological activities including memory and learning and also in neurodegeneration. The NMDARs are the ion channel forming (ionotropic) receptors composed of three subunits, NR1-3. Upon activation, NMDARs allow cations especially calcium to enter through the channel leading to activation of intracellular signaling pathways which impart physiological functions like learning and memory. However, excessive intracellular calcium influx initiates a sequence of events leading to pathological conditions like excitotoxicity. The NMDARs have complex gating mechanism which requires binding of different ligands along with cellular depolarization [7].

The NMDARs are heterotetramers consisting of two NR1 and two NR2 subunits. Together, the NR1 subunits develop the ion channel and impart the necessary features to the NMDARs including glycine activation, glutamate activation, zinc inactivation, magnesium block, *p*H sensitivity and interactions with polyamines [6]. Apart from ligand binding, depolarization of the NMDARs expressing neurons is essential to remove the resting magnesium blockage from the ion channel pore. Zinc can attenuate the glutamate elicited NMDAR current. Physiologically, magnesium is removed from the receptor channel pore upon activation of other ionotropic receptors (AMPA and kinate). Glycine is an essential co-agonist necessary for complete activation of NMDARs [8].

NR2 subunits possess refining and regulatory roles in NMDAR functioning. There are four NR2 subunits which have yet been identified ($NR2_{A-D}$). Among them $NR2_A$ are predominantly expressed in different brain regions. $NR2_B$ are distributed in the forebrain, $NR2_C$ in the cerebellum while $NR2_D$ are located in the thalamus. NR2 subunits determine the properties of NR1 subunit ion channel pores [9].

Recent findings have shown the role of a third subunit in the NMDARs family. This NR3 subunit is composed of two isoforms: $NR3_A$ and $NR3_B$. $NR3_A$ is predominantly localized in the CNS while $NR3_B$ is primarily found in the motor neurons. Reports suggest that NR1/$NR3_A$ and

$NR1/NR3_B$ subunit complexes are not responsive to glutamate or NMDA, rather they develop an excitatory calcium-impermeable response through glycine [6].

1.2. AMPA and kinate receptors

AMPA and kinate receptors are also ionotropic glutamate receptors that are named after the ligands that specifically activate them, i.e. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate). AMPA receptors are composed of four subunits ($GluR_{1-4}$) that need only glutamate to activate them. The cation permeability of AMPA receptors depends upon the subunit composition. $GluR_1$, $GluR_3$ and $GluR_4$ impart high calcium permeability and show inwardly rectifying relationship while $GluR_2$ subunit attenuates calcium entry. AMPA receptors control fast excitation which is essential to remove magnesium blockade from NMDAR [7].

Kinate receptors are composed of $GluR_{5-7}$ and KA_{1-2} subunits. The sensitivity of kinate receptors to allow ion influx on treatment with glutamate is similar to AMPA receptors, however they are generally impermeable to calcium ions. Kinate receptors are predominantly expressed postsynaptically. However, some studies suggest their presynaptic localization. Postsynaptic role of kinate receptors involves removal of magnesium block from NMDAR similar to AMPR receptors [7].

1.3. Metabotropic receptors

Metabotropic glutamate receptors (mGluRs) are single-peptide seven-transmembrane spanning proteins associated with intracellular G proteins. It has been observed that all the mGluRs use G-proteins as transduction molecules; however studies suggested that G-protein independent mechanism also exists. Presently, eight different mGluRs ($mGluR_{1-8}$) have been identified that are divided into three groups based on their sequence homology and their intracellular actions [6, 7].

Group I mGluRs comprise of $mGluR_1$ and $mGluR_5$. Activation of these receptors is mediated via G-proteins to activate phospholipase C which ultimately imparts inositol triphosphate generation and subsequent intracellular calcium metabolism. Group II mGluRs consist of $mGluR_2$ and $mGluR_3$. They attenuate adenylate cyclase signaling resulting into

inhibition of voltage-dependant calcium channels. Group III mGluRs include $mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$. They possess characteristics similar to group II mGluRs. With regard to excitotoxicity, group I mGluRs are linked to the post-synapses leading to acceleration of NMDAR-mediated calcium influx. The remaining groups of mGluRs are found pre-synaptically and abrogate calcium influx through NMDARs [10].

1.4. NMDAR-mediated excitotoxicity

The nervous system has characteristics to transfer sensory as well as motor information from one part of the body to another part, resulting in generation of thoughts and memories. The activity strongly depends upon a ubiquitous excitatory neurotransmitter, i.e. glutamate. Although the brain is dominated with other excitatory neurotransmitters, glutamate is the most common neurotransmitter with wider distribution. Most neurons (as well as glia) have high levels of glutamate (approximately 10 mM) [11]. Upon synaptic activation with sufficient magnitude, already stored glutamate inside the synaptic vesicles is released for a very short duration (milliseconds) to communicate with several other neurons through synaptic endings [12].

As glutamate is a strong excitatory neurotransmitter, its excess amount or longer exposure can induce excitotoxic cell death. There are a large number of alterations that can induce release of excess glutamate in the nervous system resulting in excitotoxic neuronal cell death. Upon mechanical injury to the nervous system, large amount of glutamate is released from the damaged cells. This excess amount of glutamate affects a number of nearby cells that have escaped from the initial injury, inducing their depolarization, swelling, lysis and finally death by apoptosis and/or necrosis. The dead cells discharge more glutamate resulting in a sequence of autodestructive programs that ultimately lead to cell death [13].

Excitotoxicity is mainly attributed to the glutamate receptor overactivation, especially the NMDARs, relieving physiological magnesium blockade with subsequent high calcium influx, leading to excitotoxicity and cell death. Excitotoxicity is responsible for many neurodegenerative disorders [6]. The increased levels of intracellular calcium, enhance lipid peroxidation of cell membrane, mitochondria and endoplasmic reticulum (ER). Lipid peroxidation also increases formation of 4-hydroxynonenal (HNE) that disturbs membrane transporters and ion channels upon peroxidation of their membrane lipids. High intracellular calcium levels also lead to

increased production of superoxide redicals (O_2^-) and nitric oxide (NO) which form peroxynitrites. Lipid peroxidation also affects the function of Na^+/K^+ATPase and glucose transporters resulting in disturbance of ionic homeostasis in the ER and mitochondria, ultimately leading to lesser supply of ATP [14].

NMDAR mediated massive influx of extracellular calcium leads to stimulation of a number of calcium dependant enzymes like phospholipase C, protein kinase C, nitric oxide synthase (NOS), phospholipase A2, memberane associated protein kinase (MAPK), calcium/calmodulin dependant protein kinase II (CaMKII), and various proteases and nucleases that lead to catabolism of phospholipids, proteins and nucleic acids thus causing cell death through different pathways [15]. For example, activation of phospholipase A2 induces extensive breakdown of membrane, while stimulation of proteases lead to characteristic cytoskeletal alteration (blebbing). Activation of phospholipase A2 followed by release of arachidonic acid leads to generation of oxygen radicals (see Fig. 1) [16].

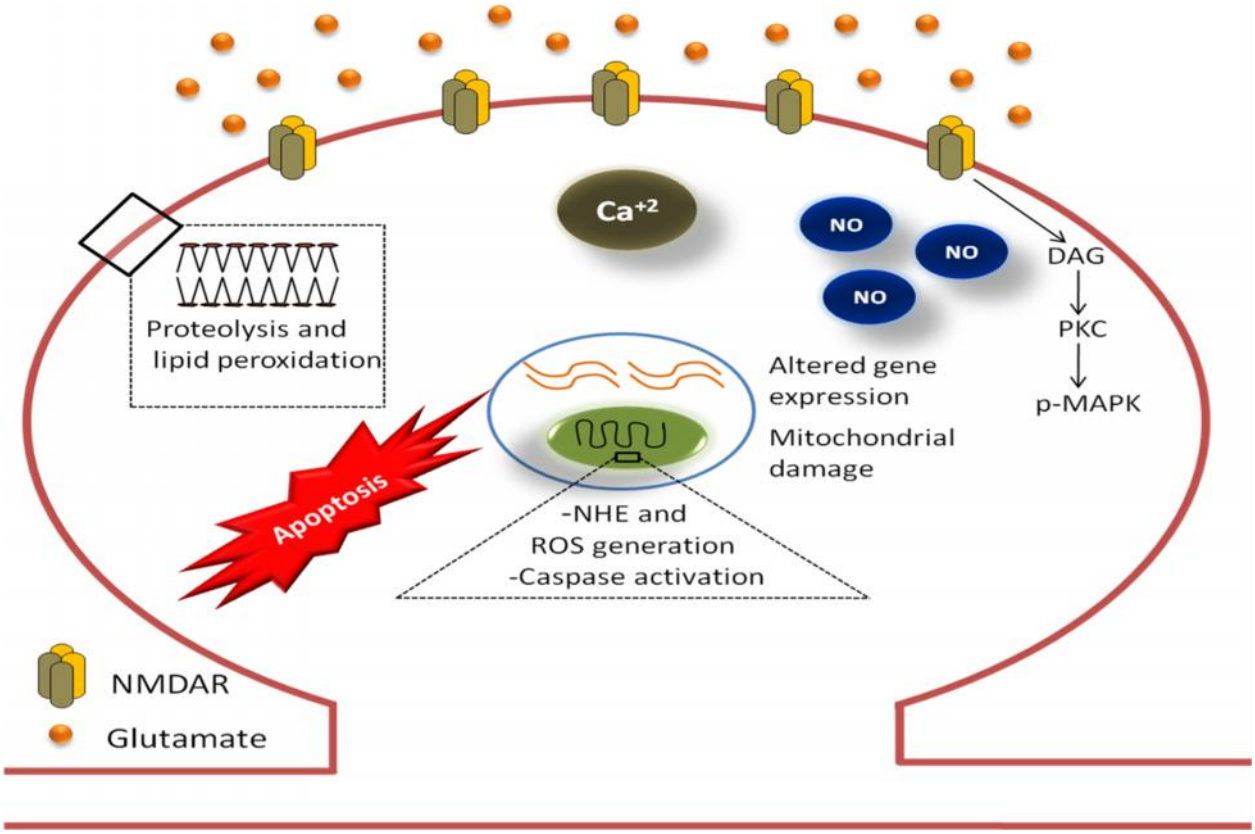

Fig. 1: Schemetic representation of cascade of events that take place during the apoptosis induced by NMDAR mediated excitotoxicity.

The role of oxidative stress in the onset and development of excitotoxic neuronal cell death has been well established. Oxidative stress alters the structures of proteins, nucleic acid molecules and lipids, that opens the mitochondrial permeability transition pores resulting in additional stimulation for production of oxygen species and release of pro-apoptotic factors [17]. It has been reported that during the NMDAR activation, ROS is generated by action of NADPH oxidase in postsynaptic neurons. Elevated ROS generation and down regulated antioxidant defense mechanisms are the most common causes resulting in neuronal cell death in neurodegenerative conditions including AD [17]. In AD, Aβ-mediated neurotoxicity is also attributed to the altered intracellular calcium levels, phosphorylation of tau protein, generation of free radicals and activation of caspase cascade leading to apoptotic cell death, which could be attenuated by free radical scavengers such as vitamine E [18].

1.5. Alzheimer's disease and excitotoxicity

As discussed earlier, excitotoxicity is a key pathological feature for the onset and development of various neurodegenerative diseases. Among them, we are mainly focusing on the AD in the present study. NMDAR overactivation is believed to be a key event in AD leading to massive calcium influx which triggers different calcium dependant signaling pathways leading to excitotoxic neuronal cell death. As discussed above, glutamate is the main excitatory neurotransmitter involved in the NMDAR overactivation-mediated excitotoxicity [13, 15].

Apart from glutamate, the role of amyloid beta (Aβ) peptide, a key pathological biomarker of AD, in the NMDAR mediated excitotoxicity has also been elucidated over the past several decades. A number of studies have revealed that Aβ peptides mediate toxic effects through several mechanisms like oxidative stress, mitochondrial dysfunction, alteration of membrane permeability, inflammation, synaptic dysfunction and excitotoxicity via interactions with different neurotransmitter receptors, channels and other membrane proteins [19]. Interaction of Aβ with different neurotransmitter receptors is considered as an important pathogenic incident in the development of AD, both in the synaptic dysfunction associated with cognitive impairment as well as in the events leading to direct neuronal injury i.e. excitotoxicity. The earliest deteriorating effects of acute exposure of Aβ on the synaptic plasticity clearly revealed that such effects are mediated by mechanisms involving immediate effect on synaptic neurotransmission. It has also been revealed that such effects could be attenuated by NMDAR antagonists

suggesting action of Aβ over the NMDARs leading to attenuation of glutamate neurotransmission [1, 19]. There are several reports which demonstrated effects of Aβ on the glutamate transport mechanisms. Studies using hippocampal slices indicated that glutamate uptake is altered in the aged hippocampus. In addition, Aβ peptides augment the uptake of glutamate, especially in aged animals. Exposure of Aβ fragments to the cultured neurons and astrocytes for 30 min elevated glutamate levels in the medium by reducing glutamate uptake. A possible mechanism responsible for such effects may involve attenuation of glutamate transporters through oxidative insult from lipid peroxidation products (like HNE) and ROS. Aβ has the ability to facilitate HNE binding with the glutamate transporters leading to attenuation of glutamate uptake [20].

There are several other reports indicating direct effect of Aβ on the NMDAR functions. Exposure to MK-801 (NMDAR antagonist), or removal of extracellular calcium attenuated Aβ-induced calcium influx, neurotoxicity and NO production in cultured neuroblastoma (MES 23.5) cells. MK-801 moderately abolished the attenuation of cell viability and energy alteration in HEK 293 cells expressing NR1/$NR2_B$ or NR1/$NR2_A$ subunits when insulted by Aβ. Several other evidences suggest that Aβ/NMDAR interactions include co-immunoprecipitation of Aβ dodecamaric oligomers with NR1 and NR2. Aβ Peptides bind with glutamatergic neurons possessing NR1 and NR2 subunits but not GABAergic neurons. Aβ attenuated both [^{3}H]glycine and [^{3}H]glutamate binding and potentiated functional [^{3}H]MK-801 binding. Decker *et al* have demonstrated that NMDAR knock-down in mature hippocampal neuronal culture attenuated Aβ oligomer binding with dendrites and thus abrogated the associated oxidative stress [21].

Reports also state that Aβ peptides have influence on NMDAR mediated long term potentiation (LTP). Exposure of Aβ to the rodent hippocampal slices strongly abolishes the generation of LTP in the dentate gyrus and CA1 regions. However, Aβ does not have effect on NMDAR independent LTP. These effects are attributed to the interaction of Aβ with NMDAR as these effects are blocked by several NMDAR antagonists [22]. Apart from that there are ample *in vivo* reports demonstrating that Aβ toxicity is attributed to the NMDAR overactivation since these effects are blocked by NMDAR antagonists. Oxidative stress elevated after the *in vivo* Aβ application, is significantly attenuated by NMDAR antagonism [23]. There are several behavioural studies supporting specific effects of Aβ on NMDAR dependant learning. Sipos *et al*

showed that bilateral injection of $A\beta_{1-42}$ into the entorhinal cortex of rats caused altered memory recognition as assessed using Morris water maze (MWM) and object recognition tasks. Similar types of behavioural alterations were observed with NMDA administration suggesting that a common mechanism could play a role. Another study showed that administration of MK-801, an NMDAR channel blocker, 2 hr prior to the $A\beta_{1-42}$ infusion into the nucleus basalis neurons attenuated passive avoidance learning deficits assessed after two weeks. Moreover, in most of the experiments memantine corrected bahaviuoral and neurochemical alterations arising after Aβ exposure or overproduction, suggesting the role of NMDAR antagonists in AD pathogenesis [22, 23].

1. INTRODUCTION

Since the finding of the physiological role of dopamine (DA) a tyrosine metabolite, more than 50 years ago, it was recognized as an important neurotransmitter in neuropharmacology. Dopaminergic innervations are the most promising ones in the human brain involved in various disorders affecting brain functions namely Parkinson's disease, attention deficit disorder, schizophrenia, drug dependence and some endocrine disorders [1, 2]. Abnormal dopaminergic signalling has also been involved in various other brain disorders like major depression, bipolar disorder, dyskinesias and somatic disorders like kidney dysfunction and hypertension [2, 3].

After being released, DA acts on various members of a family of G protein-coupled DA receptors including D_1 to D_5. Several specific DA receptor agonists and antagonists have been discovered which significantly affect the dopaminergic neurotransmission and DA-dependant functions through reinforcing or abrogating the actions of DA. Many of these active compounds have been used to treat various pathological conditions [2, 4, 5]. DA is distributed in the brain in a restricted fashion unlike noradrenaline with the highest abundance in the corpus striatum, an important part of extrapyramidal motor nervous system responsible for the coordination of movements. Also, high concentration of DA is found in some parts of the hypothalamus and limbic system [3]. DA is synthesized similar to noradrenaline from tyrosine which is converted into dopa (the rate limiting step) following decarboxylation, forming DA. Dopaminergic neurons lack DA β-hydroxylase, so they do not produce noradrenaline. DA is metabolized by two key enzymes namely monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). The key metabolites are dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HAV, a methoxy derivative of DOPAC).

1.1. Dopaminergic pathways in the CNS

1.1.1. Nigrostriatal pathway

Substantia nigra is a small area of midbrain from which components of the basal ganglia originate. It consists of two parts - the input part is called substantia nigra pars compacta (SNpc) while the output part is substantia nigra pars reticulate (SNpr). The dopaminergic neurons are abundant in the pars compacta and nearby regions. The dopaminergic neurons projecting from the substantia nigra pars compacta to the dorsal striatum is named as nigrostriatal pathway which

plays a vital role in controlling motor functions and learning newer motor skills [6]. They are highly vulnerable to damage resulting in the development of Parkinsonian syndrome [7].

1.1.2. Mesocorticolimbic pathway

The ventral tegmental area (VTA) is also an important area of midbrain. The VTA dopaminergic neurons project to the prefrontal cortex via the mesocortical pathway and another small group projects to the nucleus accumbens via the mesolimbic pathway. Collectively these two pathways are termed as mesocorticolimbic pathway. The VTA also sends dopaminergic projections to the amygdale, cingulated gyrus, hippocampus and olfactory bulb. The mesocorticolimbic pathway plays an important role in reward and other aspects of motivation [6, 7].

1.1.3. Tuberoinfundibular pathway

The arcuate nucleus and the periventricular nucleus of the hypothalamus consist of dopaminergic neurons which develop an important projection called as the tuberoinfundibular pathway which goes to the pituitary glands affecting the prolactin hormone secretion [6]. Dopamine is the primary neuroendocrine inhibitor of the prolactin secretion from the anterior pituitary gland [7].

1.2. Classification of DA receptors

Presence of DA receptors in the brain evidenced first in 1972 from the biochemical studies revealed that DA can stimulate adenylate cyclase (AC) [4]. In 1978, DA receptors were introduced on the basis of biochemical and pharmacological evidences, as two different subtypes.

As discussed earlier, DA mediates its pharmacological actions via five distinct but closely linked G-protein coupled receptors (GPCRs) which are classified into two groups: D_1-like and D_2-like family of receptors [8]. This classification has arisen from the initial biochemical observations revealing the ability of DA to modulate AC activity. In this pioneering work, it was shown that only one subtype of DA receptors was positively linked to AC. Based on this finding it was speculated that DA receptors could be present into two different groups. Subsequently it led to the categorization into D_1 and D_2 subtypes of DA receptors, mostly on the

basis of their ability to change cAMP production and their distinct pharmacological properties [4]. Later on, the description of the DA receptor families based upon the genetic cloning approach exposed that multiple DA receptor subtypes are activated by DA. Based on the structural, biochemical and pharmacological properties, these receptors were divided into D_1-like and D_2-like DA receptor families. D_1-like family comprises of D_1 and D_5 receptors while D_2-like family comprises of D_2, D_3 and D_4 receptors [8].

Individual members of the DA receptor subfamilies share high degree of homology in their transmemberane domains with distinct pharmacological properties. It has been well documented that D_1 class of DA receptors activates the $G_{\alpha s/olf}$ proteins to activate the cAMP production through AC and are expressed selectively postsynaptically on DA receptor expressing cells including striatal GABA-ergic medium spiny neurons (MSNs). The D_2 class DA receptors are coupled to $G_{\alpha i/o}$ proteins and therefore attenuate cAMP production through abrogation of AC. Opposite to D_1 family receptors, D_2 family receptors are present both presynaptically and postsynaptically [2, 9].

D_1 and D_2 class DA receptors have distinct genetic structures, mainly with respect to the existence of introns in the coding region. The genes of D_1 class receptors have no introns in the coding sequence; however D_2 family receptors contain quite a few introns, with six introns in the D_2 receptor encoding gene, five in the D_3 receptor encoding gene and three in the D_4 receptor encoding gene [2, 5]. Thus, the D_2 class of receptors are enabled to generate the receptor splice variants due to their characteristic genetic organization. An 87-base-pair axon of the D_2 receptor undergoes alternate splicing between introns 4 and 5 that leads to generate two D_2 receptor variants which are termed as D_2-short (D_{2S}) and D_2-long (D_{2L}). These two D_2 receptor variants have different anatomical, pharmacological, physiological and signalling properties [10].

The D_1 class DA receptors have different structural and genetic characteristics. The D_1 family receptors share 80 % homology in the transmembrane domains, while the D_3 and D_4 receptors share 75 and 53 % sequence homology respectively, with D_2 receptor. In the NH_2-terminal part, all the DA receptors have the same amino acids whereas, the COOH-terminal part of D_1 family receptors is seven times longer than the D_2 family of receptors [5, 8].

DA activates different DA receptors with distinct binding affinity varying from micromolar to nanomolar concentrations. Thus, distinct DA receptor subtypes have different sensitivity for DA receptor agonists and antagonists. Over the past several years, a number of DA receptor agonists and antagonists have been developed to treat various neuropathological conditions. The D_4 receptor antagonists possess the highest selectivity, showing more than 1000 fold selectivity in comparison to other DA receptor subtypes [9, 11].

1.3. DA receptor expression

DA receptors are widely expressed in the CNS as well as peripheral nervous system (PNS). The brain distribution of the D_1 receptors is seen in the mesolimbic, nigrostriatal, and mesocortical parts including the nucleus accumbens, striatum, olfactory bulb, substantia nigra, frontal cortex and amygdale. D_1 receptors are also expressed to a lesser extent in the cerebellum, hippocampus, hypothalamic and thalamic parts. The D_5 receptors are highly expressed in the medium spiny neurons (MSNs) of the caudate nucleus and nucleus accumbens. The least expression of D_5 receptors is observed in different brain parts such as prefrontal cortex, entorhinal cortex, substantia nigra, hypothalamus, hippocampus and dentate gyrus [2, 5, 8, 9].

D_2 receptors are highly expressed in the nucleus accumbens, striatum and the olfactory tubercle. D_2 receptors are also seen in the hypothalamus, hippocampus, substantia nigra, cortical areas, ventral tegmental area, amygdale and septum [4, 5, 8]. The D_3 receptors have limited distribution with the highest expression in the limbic parts including the olfactory tubercle, nucleus accumbens, and islands of Calleja. Also a lower level of D_3 receptors is observed in the hippocampus, striatum, ventral tegmental area, septal area, substantia nigra pars compacta and several cortical regions [12]. The D_4 receptors have the least presence in the brain with significant amounts in the hippocampus, frontal cortex, hypothalamus, amygdala, thalamus, globus pallidus and substantia nigra pars reticulate [5, 8, 11].

D_1, D_2 and D_4 receptors are also present in the retina, with predominant expression of D_2 receptors in the pituitary gland. Apart from CNS, DA receptors are also distributed in the periphery including kidney, gastrointestinal tract, adrenal glands, blood vessels, sympathetic ganglia and heart [2, 3].

1.4. Functions of DA receptors

DA is critically involved in various physiological activities; therefore functions of various DA receptor subtypes have been broadly elucidated. The main investigated function of DA receptors is in regulation of locomotion. Several reports suggest that locomotor activity is mainly regulated by D_1, D_2 and D_3 receptors. The exclusively postsynaptically located D_1 receptors have a mild effect potentiating locomotor effect. The effects of D_2 and D_3 receptors are more complicated as compared to D_1 receptors due to their presynaptic as well as postsynaptic localization [5, 8, 13].

Presynaptic autoreceptors impart a vital negative feedback to control neuronal synthesis, release and firing rate of the neurotransmitters in response to alteration of extracellular neurotransmitter levels [13]. The presynaptic D_2 family autoreceptor activation decreases DA firing resulting in reduced locomotor activity. In contrast, postsynaptic activation of D_2 class of receptors potentiates locomotor activity. D_2 family of autoreceptors are activated by somewhat low levels of DA agonists in comparison to the concentrations required to stimulate receptors located postsynaptically. Therefore, an identical DA agonist can produce a biphasic response, producing reduced activity at lower concentrations while behavioural activation at higher doses. Among the D_2 class of receptors, D_2 receptors are the predominant autoreceptors regulating presynaptic synthesis, release and firing rate of DA. As discussed earlier, D_2 receptors have two splice variants, D_{2L} and D_{2S}, having different neuronal distribution. D_{2L} is predominantly located presynaptically while D_{2S} is located postsynaptically. Thus, the distinct effects of presynaptic and postsynaptic D_2 receptors depend upon the degree of contribution of these isoforms. D_3 receptors impart mild effect, inhibiting locomotor activity either through autoreceptor action or via the postsynaptic action [12, 13]. Because of limited pattern of distribution in the primary motor regions of the brain, D_4 and D_5 receptors have minimum effect in controlling the movement [5, 11, 13]. Most importantly, it is clear that both the D_1 and D_2 classes of DA receptors located postsynaptically are essential for the locomotor activity.

There are several other important effects which require DA receptor activation in the brain. D_1, D_2 and also to some extent, D_3 receptors are useful in the reinforcement and reward mechanisms. Therefore DA receptors are considered as an important topic to be studied in the drug addiction research [1, 5]. D_1 and D_2 receptors play a pivotal role in memory and learning,

including working memory which is primarily mediated through prefrontal cortex [8]. In contrast, D_3, D_4 and D_5 receptors have the least modulatory effect in cognitive mechanism which is primarily controlled by the hippocampus [5, 11, 12]. It has also been elucidated that essentially all clinically used antipsychotic drugs antagonise D_2 receptors indicating that D_2 receptors have an important position in the psychiatric diseases like bipolar depression and schizophrenia [8]. There are several other functions which are being governed to some extent through different DA receptors that include motor learning, attention, sleep, impulse control, food intake, decision making and reproductive behaviour [1-3]. Other functions being regulated by the DA receptors located outside the CNS include vision, olfaction, and hormonal control like, the D_2 receptors in pituitary gland regulate prolactin secretion, kidney D_1 receptors regulate rennin secretion and adrenal gland D_2 receptors control aldosterone secretion. D_1, D_2 and D_4 receptors also control blood pressure, renal function, gastrointestinal motility and vasodilation [2, 3, 5, 8].

1.5. Parkinson's disease

Parkinson's disease (PD) is the most common neurodegenerative disorder. It is an age related disorder with the prevalence of 1-2 % in people over the age of 50 years. It is also known as a motor disorder with the appearance of complications like rigidity, bradykinesia, postural instability and resting tremors [14]. The main pathological characteristic of PD is the degradation of dopaminergic neurons from the SNpc associated with Lewy bodies, which are the intracellular protein aggregates resulting in reduced striatal DA concentration [15]. Estimations suggest that at the onset of symptoms about 70 % dopaminergic input is lost, and at post-mortem examination, the depletion would exceed 90 %. Using modern pharmacological therapy, patients suffering with PD are approaching life expectancy similar to those who do not contract the disease. However, the quality of life is greatly diminished along with economic burden [14].

1.5.1. Oxidative stress in PD

In PD pathogenesis, oxidative stress is considered as the most common pathological feature that develops cellular alterations and ultimately cell death. Patients of PD, exhibit elevated lipid peroxidation, decreased glutathione (GSH) levels and increased superoxide activity in substantia nigra. Generally, oxidative stress results due to an imbalance between production of reactive oxygen species (ROS) and cellular antioxidant activities. Dopaminergic

neurons have predominant abundance of ROS-developing enzymes including monoamine oxidase (MAO) and tyrosine hydroxylase (TH), subjecting them more vulnerable to oxidative stress. Moreover, the dopaminergic neurons in the substentia nigra contain iron that plays a role in the Fenton reaction, where superoxide radicals further contribute to oxidative stress. Thus, due to intrinsic sensitivity of nigral dopaminergic neurons for reactive oxygen species, a mild oxidative stress may initiate a sequence of events leading to the cell death [16]. There are several factors responsible for causing oxidative stress in the nigral dopaminergic neurons.

DA metabolism is the major factor in triggering oxidative stress. Reports suggest that DA oxidation and consequent quinone modification develops oxidative stress making dopaminergic neurons more susceptible to the oxidative damage. Outside the DA storage vesicles, DA is readily oxidized either spontaneously or enzymatically to form DA quinone. DA quinone species are able to alter various cellular proteins which are responsible for cell survival. DA quinone can also affect a number of proteins whose dysfunction is involved in PD pathogenesis like parkin, α-synuclein, UCH-L1 and DJ-1 [17].

Mitochondrial dysfunction is also an important source of oxidative stress in PD. In normal physiology, neurons acquire aerobic respiration during which superoxide radicals are generated in the mitochondria as byproducts. However, in pathological conditions, mitochondrial dysfunction can induce elevated ROS generation which beats the cellular antioxidant mechanism. Oxidative stress induces phosphorylation of the mithochondria-specific lipid cardiolipin, resulting in release of cytochrome c in the cytosol which ultimately triggers apoptotic cell death. As stated above, dopaminergic neurons are intrinsically more vulnerable to oxidative damage; any incident causing further oxidative stress is dangerous for the cell [16].

1.5.2. Direct and indirect pathways

The basal ganglia comprise of the striatum subthalamic nucleus (STN), internal and external globus pallidus (GPi and GPe, respectively) and SNpc and SNr. It has been well established that the functions of these areas are mainly controlled by the neurotransmitter DA. The basal ganglia components are involved in different functionally and anatomically segregated circuits. Among them, motor circuit arises from the frontal cortex motor region, comprising of

motor parts of the striatum including, GPe, GPi, STN and thalamus, and finally returns to the frontal cortex [18].

It has been speculated that coordinated movements are controlled by two parallel and segregated pathways in the basal ganglia which are composed of GABAergic medium spiny neurons (MSNs) of the striatum. The MSNs present in the striatum are divided into two subtypes- MSNs having D_1 receptors and MSNs having D_2 receptors.

- The D_1 receptor expressing MSNs projects directly to the output nucleus of the basal ganglia i.e. GPi/SNr. It is termed as D_1 pathway or direct pathway.
- The MSNs having D_2 receptors project initially to the GPe which then sends inhibitory projections to the STN. The STN finally projects to the output nucleus of the basal ganglia including GPi/SNr. It is termed as D_2 pathway or indirect pathway.

Activation of D_1 receptors on direct pathway neurons facilitates corticostriatal transmission. In contrast, activation of D_2 receptors on indirect pathway neurons reduces this transmission. The coordinated outflow from these two pathways causes decrease in activity of GPi/SNr neurons, and enables motor activity through disinhibition of thalamocortical circuit [15, 18].

1. INTRODUCTION

Dopamine (DA), an endogenous neurotransmitter of the central nervous system (CNS), mediates its actions via two DA receptor families namely D_1-like and D_2-like families of receptors. D_2-like family comprises of D_2, D_3 and D_4 receptors which exhibit approximately 40% structural homology amongst themselves. DA receptors play a pivotal role in the pathogenesis of neuropsychiatric disorders including Parkinson's disease (PD) and schizophrenia [1]. The classification and functions of different DA receptors and various dopaminergic pathways in the CNS have been discussed in Chapter 4.

Schizophrenia is an overwhelming mental sickness that alters social and mental functioning and frequently develops many co-morbid diseases. It affects more than 21 million peoples worldwide [2]. Schizophrenia is characterised by positive and negative symptoms which affects a patient's behaviour, speech, thoughts and perceptions. Positive symptoms comprise hallucination; voices that communicate with the patient, and delusions. Negative symptoms comprise flat expression, loss of will and drive, loss of sense of pleasure, social withdrawal and lack of motivation. Schizophrenia also includes disorganized thoughts, which are manifested in behaviour and speech. The disorganized speech is commonly referred to as schizophasia (also known as "word salad"). Schizophasia is a confused and repetitive speech that uses words having no apparent meaning and relationship to one another. Disorganized behaviour may develop difficulty in performing daily living activities. Additionally, altered cognitive abilities are also considered as a key pathological feature in schizophrenia [3].

It has been reported that dopaminergic system plays a pivotal role in the regulation of neuropsychiatric conditions like schizophrenia [4]. Currently available antipsychotic treatments target mainly D_2 receptors. Haloperidol, a typical example of classical D_2 receptor antagonist, shows promising effects in controlling positive symptoms of schizophrenia [5]. However, selective D_2 receptor blockade has little effects on negative and cognitive symptoms of schizophrenia and impairs motor functions by producing extrapyramidal (motor and endocrine) side effects [6]. Hence, treatment with selective D_2 receptor antagonists results in poor patient compliance and consequently a poor psychosis control. In contrasts to this, selective D_3 receptor blockade imparts potent antipsychotic effects and facilitates motor functions.

The very first idea of using selective D_3 receptor antagonists clinically in the management of schizophrenia arose by the finding of the anatomical distribution of D_3 receptors in the brain. The expression of the D_3 receptors in the brain is less compared to the D_2 receptors. Limbic region of striatum, islands of Calleja and nucleus accumbens are the brain regions with the highest postsynaptic expression of D_3 receptors [7].

D_3 receptor signal transduction mechanisms are identical to that of D_2 receptors but their cerebral expression differs. These characteristic features impart them contrasting physiological and therapeutic properties suggesting possible therapeutic potential of the D_3 receptors in several disorders. A large number of data are available revealing the role of D_3 receptor antagonists in the management of schizophrenia suggesting their potential usage as antipsychotic agents [8-10].

Many researchers have suggested the role of limbic regions of the striatum in gating the altered neuronal process in schizophrenia due to abundant expression of D_3 receptors in the mesolimbic dopaminergic system [11]. Post-mortem studies have revealed elevated levels of D_3 receptors in schizophrenia patients which supported the above hypothesis [11]. The elevated level of D_3 receptors may develop the hyperdopaminergic condition in the mesolimbic dopaminergic system which is well characterised in schizophrenia. The hyperdopaminergic state which is elicited by *L*-dopa treatment to DA-depleted rats or in dopamine transporter (DAT) knockout mice has shown increased extracellular DA concentration and an overexpression of D_3 receptors without simultaneous increase in D_1 or D_2 receptors. D_3 receptor expression could be controlled by non-dopaminergic signalling like brain-derived neurotropic factor (BDNF) [12]. With increasing dopaminergic activity, BDNF release is elevated from corticostriatal fibres with concomitant rise in D_3 receptor level in nucleus accumbence [12]. Moreover, polymorphism of D_3 receptor Ser9Gly might be linked to an elevated risk of schizophrenia in certain patients [13].

The exact role of D_3 receptor in controlling motor behaviour is still unclear. It has been well documented that selective inactivation of D_2 receptor produces a disruptive or inhibitory effect on motor function and develops an extrapyramidal (motor and endocrine) syndrome. Opposite to it, selective blockade of D_3 receptor does not produce such effects. Selective D_3 receptor blockade demonstrates opposite though controversial effects as compared to the D_2 receptor antagonism favouring motor functions in experimental rodent and primate models [14].

Therefore, a proportionally high degree of D_3 versus D_2 receptor antagonism is favourable for low extrapyramidal potential.

Additionally, D_3 antagonism is believed to control deficit symptoms in schizophrenia. Schizophrenia symptoms are classified in positive (abnormal thoughts and perception) and negative symptoms (loss or decrease of normal functions). As discussed earlier, D_3 antagonists are least prone to induce marked extrapyramidal side effects which is an essential condition to treat negative symptoms of schizophrenia, that could not be achieved with D_2 antagonism. Depressed mood is very common in schizophrenia patients which can further develop into negative symptoms. D_2 receptor activation imparts a positive influence on depressed mood, therefore potent D_2 antagonists could have poor efficacy in controlling negative symptoms. There is no evidence of negative effects of D_3 blockade on mood. It is very difficult to reproduce negative symptoms in rodents. However, social interaction is a useful approach to evaluate negative symptoms which are intensely altered in schizophrenia patients. Different experimental rodent models have shown increased social interaction with selective D_3 blockage but not with D_2. Such observations suggest that selective D_3 antagonists could improve negative symptoms in schizophrenia patients.

Thus, a recent approach is to design potent and relatively selective D_3 antagonists for the treatment of neuropsychiatric conditions with low incidence of extrapyramidal side effects. As discussed earlier, the D_3 receptors are fewer in number in the body as compared to other D_2 family members, but have a dominant presence in brain regions (limbic region of striatum, nucleus accumbens and the islands of Calleja) which are considered as the centres for emotion, memory and motor functions [7].