Section-III

Chapter-1

INTRODUCTION

1. INTRODUCTION

Dopamine (DA) is a basic neurotransmitter in the mammalian central nervous system (CNS). The cerebral dopaminergic system is involved in the pathophysiology of a few neurobehavioral problems, including Parkinson's disease and other development and hyperactivity issues, schizophrenia, depression, mania, substance misuse and dietary issues. DA contributes importantly to the neurophysiological control of stimulation and attention, initiation of movement, motivation, perception and emotion. DA actions are intervened by five major DA receptor subtypes (D_1–D_5) with diverse differences in their gene and peptide composition, neuropharmacology and molecular functions. These receptors characterize rational targets for development of both radioligands and drugs. In recent years, substantial efforts have been focussed at the more recently described DA receptor types, D_3, D_4, and D_5, as well as the longer-known and more abundant D_1 and D_2 receptors. Current patterns in medicinal chemistry and neuropharmacology incorporate advancements of D_1 full agonists and D_2 partial agonists and in addition with dopaminergic action, with mutual effects on CNS serotonergic, muscarinic, adrenergic, and histaminic receptors.

1.1 Dopaminergic system: synthesis and degeneration of dopamine

DA is one of the characteristically happening catecholamines biosynthesized in dopaminergic neuron terminals through enzymatic pathways from the fundamental amino acid *L*-tyrosine (Tyr). Proteins in charge of this biosynthetic pathway incorporate the cytosolic catalyst tyrosine hydroxylase (TH), which changes tyrosine into *L*-dihydroxyphenylalanine (*L*-DOPA), and aromatic *L*-amino acid decarboxylase (AADC), which decarboxylates *L*-DOPA to dopamine (DA). TH is the rate-constraining enzyme that controls the synthesis of DA. Activation of this catalyst by phosphorylation through protein kinases can upgrade DA synthesis [1-3].

There is some metabolic inactivation of DA by generally extraneuronal O-methylation by catechol-O-methyltransferase (COMT). DA transported backside into presynaptic neuronal terminals and not restored in presynaptic vesicles can be oxidatively catabolized to a byproduct (3,4-dihydroxyphenylacetaldehyde [DHPA], which is quickly oxidized to 3,4-dihydroxyphenylacetic acid [DOPAC]) by intraneuronal mitochondrial monoamine oxidase (MAO), and secondly 3-O-methylated to 3-methoxy-4-hydroxyphenylacetic acid (MHPA) to finish up chiefly as the 3-O-methylated and deaminated acidic metabolite homovanillic acid (HVA). Smaller amounts of 3-methoxytyramine (3-MT) are also produced and consequently deaminated by MAO and aldehyde reductase to make additional HVA. Some DA occupied

into presynaptic dopaminergic nerve terminals is put away again in vesicles and reused. DOPAC and HVA are the real metabolites of DA that disperse into the blood and cerebrospinal fluid (CSF), and are inevitably discharged through the kidneys. Centralizations of DOPAC and HVA have been examined in plasma or CSF to screen DA digestion system and of dopaminergic dysfunction [4].

1.2 Dopaminergic neuronal pathways

There are four real DA pathways in the mammalian CNS, namely the mesolimbic, mesocortical, tuberoinfundibular and nigrostriatal pathways [5, 6]. The mesocortical framework is a neural pathway associating the ventral tegmentum of midbrain to the cerebral cortex, especially the mesioprefrontal projections. It is crucial to the typical cognitive capacity of the dorsolateral prefrontal cortex and included in inspiration and enthusiastic reaction. The mesolimbic framework is one of the neural pathways in the brain joining the ventral tegmental region (VTA) to the nucleus accumbens septi (NAS) in the limbic framework. It is included in delivering gratifying impacts connected with prize and yearning, especially owing to the association with the nucleus accumbens [7]. The nigrostriatal pathway is a neural path interfacing the substantia nigra standards compressed with the caudate-putamen in the striatum. This framework holds 70% of the DA in cerebrum tissue. Idiopathic misfortune of DA neurons in the caudate-putamen is a real neurotic peculiarity of Parkinson's disease. The tuberoinfundibular pathway found between the hypothalamus (arcuate core) and the average prominence of hypothalamus to convey DA to the tuberoinfundibular microvascular framework to be conveyed to the foremost pituitary as an administrative neurohormone. Some antipsychotic drugs acts on D_2-like DA receptors on mammotrophic cells of the front pituitary to build blood prolactin levels.

1.3 Subtypes of dopamine receptors

DA receptors belong to a superfamily of huge proteins differentiated by having seven comparatively hydrophobic fragments that are supposed to be cell-membrane spanning. They are coupled to G proteins that interact with several membrane or cytoplasmic effector molecules (usually enzymes, ion channels or transporters) that control neuronal tasks. In 1979, Kebabian and Calne [8] anticipated that DA exerts its actions by binding to two hypothetic chief receptor types, selected as D_1 and D_2 receptors. These receptors were assumed primarily based on molecular neuropharmacological data, long before their anatomical localization by receptor-selective radioligands and ultimate cloning and chemical categorization by the methods of molecular genetics [9]. The D_1 receptor was recognized primarily as interceding the stimulation of adenylyl cyclase by DA to increase creation of

cyclic-AMP. Afterwards, it was categorized by selective taging with the radiolabeled D_1 antagonist SCH-23390. A second DA receptor type was assumed, based mainly on the capability of some antipsychotic drugs, such as spiperone and haloperidol, to antagonize behavioral and metabolic actions of DA and effects of DA agonists, including *R*-(−)-apomorphine, devoid of blocking production of cyclic-AMP by DA. Selective radiolabeling of these ligands further enthused recognition of the D_2 receptor and categorization of its anatomical allocation in brain tissue [10]. Both types of DA receptors exert their biological effects by coupling to and activating diverse G protein composites. The D_1 receptor connects with guanosine triphosphate (GTP) tying proteins of the Gs type to activate adenylyl cyclase and animate amalgamation of the intracellular second messenger cyclic-AMP, although the D_2 receptor is currently identified to interface with Gi or Go proteins to repress adenylyl cyclase and additionally to inactivate Ca^{2+} currents and stimulate receptor-gated K^+ currents [11]. The anatomical conveyance of these two DA receptors in the CNS covers in quantitative proportions that vary among specific anatomical zones. In the late 1980s, provision of gene cloning and recombinant DNA technologies uncovered that there were no less than five unique DA receptors (D_1−D_5) and their sub-atomic variants. This group of DA receptors bears numerous similitudes to receptor proteins for other monoamine neurotransmitters (norepinephrine and serotonin). In people, DA receptors go in peptide length from 414 (D_2 type) to 515 amino acids [D_4 (10 with 10 repeats of a 16-amino acid succession in intracellular peptide loop 3)], with 446 in D_1, and 443 in D_2-long, the two most abundant DA receptors. The first characterization of DA receptors into two fundamental sorts, now considered D_1-like and D_2-like, still stands [12]. The D_1-like receptors incorporate D_1 (or D_{1A}) and a low-wealth D_5 (or D_{1B}) subtype. The D_2-like receptors incorporate three fundamental sorts: the most rich D_2 and in addition less basic D_3 and D_4 types, which represent a D_2-like family. The D_2 receptor in a few animal varieties additionally has two gene-join variants, a plentiful D_2-long sort and a far less regular D_2-short form [13, 14]. The endogenous ligand DA is more viable in fortifying the D_2-short structure by empowering the coupling of GTP to the receptor-partnered Gi and Go proteins [15, 16]. In man, nonhuman primates, and some different species, D_4 receptors likewise differ in their sub-atomic organization, in view of the amount of repeats of a 16-amino acid grouping found in the third intracellular circle of the receptor peptide arrangement. This succession and additionally the intra-cytoplasmic C-terminal fragment are thought to be especially discriminating for DA-

invigorated connections with G-proteins and effectors along these lines for DA receptors' functioning.

1.4 Importance of D_2 receptor

The D_2-like receptor family (D_2, D_3, and D_4) is presently of huge therapeutic interest, together with their recognition as primary sites of action of most antipsychotic and anti-Parkinsonian drugs. The D_2 receptor is the second richest DA receptor type in mammalian forebrain. It is highly expressed in the pituitary, corpus striatum, and olfactory tubercle, with lower levels in anterior cingulated, thalamus, and retrosplenial cortex. The D_2 receptor has been targeted effectively by an increasing number of agonists that reduce the rigidity, bradykinesia, and tremor characteristic of Parkinson's disease, as well as symptoms of other movement disorders, including Ekbom's restless legs syndrome [17-19]. Besides the direct precursor of DA and *L*-DOPA, D_2 full or partial agonists, including *R*-(−)-apomorphine, pramipexole, and ropinirole, are broadly utilized as treatments of Parkinson's disease. D_2 receptors are also supposed to mediate the reinforcing, dependency-producing effects of a range of different drugs of abuse. Reinforcing effects of alcohol and morphine self administration are reduced in D_2 receptor gene knockout mice and by pre-treatment with D_2 antagonists [20-23]. Medications of psychotic disorders such as schizophrenia include traditional neuroleptics (e.g., fluphenazine, haloperidol, chlorpromazine) established in the 1950s and modern, atypical, or "second generation" antipsychotics (e.g., aripiprazole, clozapine, iloperidone, olanzapine, lurasidone, quetiapine, paliperidone, risperidone and ziprasidone) [24-27]. Both types of antipsychotics have broad efficacy in the treatment of schizophrenia, mania, and other psychotic disorders, but the modern agents are usually less potent D_2 antagonists and more potent antagonists of serotonin 5-HT_{2A} (5-hydroxytryptamine) receptors, and they cause less possibility of adverse acute and late extrapyramidal neurological effects. Modern antipsychotic drugs are approximately as efficient as the classic neuroleptics but with inadequate or dissimilar safety concerns. Clozapine is special in having greater efficacy and larger potential toxicity. Many of these drugs also have acts on serotonin 5-HT_{1A} receptors as well as on glutaminergic, adrenergic, histaminergic, and acetylcholinergic neurotransmission [28-31].

1.5 Parkinson's disease (PD)

It is a chronic, progressive, neurodegenerative disorder with an estimated occurrence of 31 to 328 per 100,000 people worldwide [32]. A registry of all cases of PD in northern Manhattan from 1988-1993 showed an occurrence rate of 107 per 100,000 people [33]. It is

assessed that about 1 to 2 % of the population over an age of 65 have PD [34]; incidence and occurrence increase with age [32, 35]. With the increase in the average age of the population of western countries, an increase in the incidence of PD is to be ordinary. Several studies states that PD affects males and females uniformly, while others states that PD is somewhat more common in men [32, 33]. All races and tribal groups are affected [32]. The highest reported occurrences are in Caucasians, and the lowest in African blacks and Asians [36]. The incidence of PD is stated to be the highest in Europe and North America, and the lowest in Africa, Japan and China [32, 36], although lack of standardized diagnostic criteria weakens the capability to amass accurate occurrence rates [34]. The mortality for elderly PD patients is 2 to 5 times higher than in age-matched controls [37]. The total annual cost for PD in the United States is estimated to be approximately $26 billion, including direct and indirect costs and lost productivity [38]. Clearly, PD places a main burden on both individual and communal healthcare funds [39].

1.5.1 Pathology, aetiology and pathogenesis

The pathological characteristic of PD is cell loss within the substantia nigra mainly, affecting the ventral part of the pars compacta. By the time of death, this area of the brain has lost 50–70% of its neurons compared with the same area in unaffected individuals. The earliest documented pathological changes in PD have been observed in the olfactory bulb and medulla oblongata/pontine tegmentum. In these early stages—Braak stages 1 and 2—patients are pre-symptomatic. As the disease advances—Braak stages 3 and 4—the substantia nigra, areas of the midbrain and basal forebrain become occupied. Finally, the pathological alterations emerge in the neocortex. This pathological staging is based on the allocation of Lewy bodies. Lewy bodies are the pathological characteristic of PD. They are a-synuclein-immunoreactive insertions made up of a number of neurofilament proteins together with proteins accountable for proteolysis (**Figure 1**). These include ubiquitin, a heat shock protein which plays an essential role in targeting other proteins for breakdown. Mutations in the a-synuclein gene are accountable for some familial forms of PD in which Lewy bodies are also seen. Mutations in the Parkin protein produce a Parkinsonian syndrome without Lewy bodies in adolescent cases suggesting that the Parkin protein plays an essential role in the growth of the Lewy body. It has been shown that Parkin assists the binding of ubiquitin (ubiquination) to other proteins such as the a-synuclein interacting protein synphilin-1 leading to the development of Lewy bodies [40]. Lewy bodies are found in dementia and PD with Lewy Bodies (DLB), but are not a pathological characteristic of any other neurodegenerative disease. The recognition of single gene defects in PD has determined concern on the

ubiquitin-proteosome system (UPS) as one possible contestant in the development of cell death [41]. The UPS is essential for intracellular proteolysis and a large number of intracellular progressions that sustain the viability of cells. It does this by removing useless proteins that are no longer required by the cell. Breakdown of the UPS leads to the anomalous aggregation of proteins including a-synuclein which is a key component of Lewy bodies. One of the first sites for LB deposition in early PD is the olfactory bulb. It is, therefore, of interest that a trouble in taste and smell is often one of the most primitive clinical features in PD raising the likelihood that LB formation may be vital for the activation of pathways leading to neuronal dysfunction and death. The link between UPS and neurodegeneration has been toughened by the discovery of mutations in genes which code for several ubiquitin-proteosome pathway proteins in PD.

1.5.2. Genetics in PD [42, 43]

Although PD is usually a periodic disease, there are a rising number of single gene mutations which have been recognized. At the time of writing, 11 genes have been mapped by genetic linkage with six genes recognized- ubiquitin C-terminal hydrolase like 1 (UCH-L1), a-synuclein (SNCA), Parkin (PRKN), PINK 1, LRRK 2 and DJ-1 genes. These single gene defects with the remarkable exemption of LRRK 2 are accountable for only a small number of patients with PD, though more importantly their detection and the proteins that they encode for are providing significant insight into the disease mechanisms that may be accountable for PD and other neurodegenerative diseases (**Figure 1**). A point mutation of the SNCA gene leads to the early onset of PD in concerned members in an autosomal dominant pattern. Of interest, duplication or triplication of the SNCA gene in concerned members' leads to PD symptoms developing at a later age in the 4^{th} or 5^{th} decades raising the possibility that overexpression of SNCA may be a factor in periodic disease. The LRRK 2 gene (PARK8) is the most widespread cause of familial or the so-called 'sporadic' PD to date [44]. The frequency of LRRK2 mutations in patients with a family history of PD is 5–7%. The heterozygous mutation, 2877510 g -->A, produces a glycine to serine amino acid substitution at codon 2019 (Gly2019 ser). This LRRK2 G2019S mutation is described most commonly, accounting for the majority of familial cases and up to 1.6% of cases of idiopathic PD, though the occurrence seems to be variable. The LRRK2 gene encodes for a protein named dardarin (derived from the Spanish word for tremor; the original families described came from Spain and England). Lewy bodies have been identified in some LRRK 2 cases. Many of the accounted LRRK2 patients have typical features of PD with onset in middle or late onset. Symptoms at onset may be typical of idiopathic PD categorized by rigidity and unilateral

bradykinesia, with tremors present in some but not all patients. A number of single gene mutations, e.g. Parkin and DJ-1 with an autosomal recessive pattern of inheritance, may have a clinical example of earlier age of onset, a more benign course with good response to levodopa and the presence of dystonia.

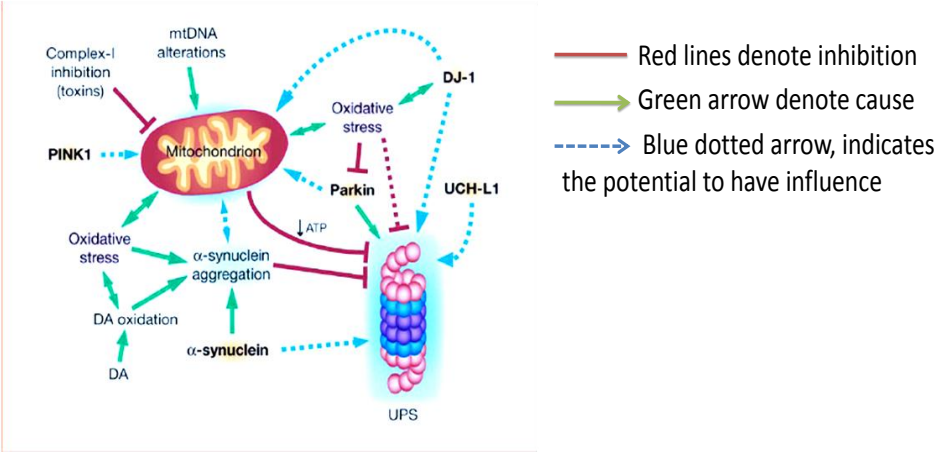

Figure 1: Aggregation of α-synuclein evidently reduced from complex-1 inhibition and aggregation similar to that could also inhibit proteasomal function. If inhibition of complex-1 is the centre of pathogenesis of PD, then the chain of events triggered by α-synuclein aggregation enhanced oxidative stress and ATP synthesis deficit, all of which can interfere with normal function of UPS. UPS will result in inhibition of protein gathering in addition targeted for degradation, some of which are cytotoxic, which in combination with oxidative risk will surely result in death of dopaminergic neurons. Parkin, UCH-L1, and DJ-1 are involved in maintaining UPS, while PINK-1 along with Parkin and DJ-1 would control the normal function of mitochondrion. Disease correlated mutations in these genes will lead to a group of events that started the death of dopaminergic neurons. However, this incident path can cause proteasomal inhibition but can also go backwards and forwards disrupting mitochondrion function. This study leads to large degree of cross correlation between mitochondrion and UPS, and dysfunction in each or all of the system will lead to a familiar end point of degeneration of DA neurons.

However, it is not likely to categorize Parkin positive young onset PD patients from Parkin negative patients on clinical features alone. There has been a great deal of research into mitochondrial genetics and function in PD. Deformities in Complex 1 of the oxidative phosphorylation enzyme pathway is the most reliable finding, having been detected in PD blood platelets, brains and skeletal muscles, although defects in other tissues have also been accounted [45]. It appears that the cells of the pars compacta are mainly susceptible to oxidative damage. Mitochondrial DNA studies have as yet failed to recognize a compelling gene mutation to describe the oxidative phosphorylation defects in PD. However, it seems likely that a mitochondrial defect may play a part in the pathways leading to cell dysfunction and death. The PINK1 gene codes for a mitochondrial complex and has been shown to be

responsible for an autosomal recessive form of PD, though is not a major risk factor for sporadic disease.

1.5.3. Diagnosis

Plenty of guidelines for PD diagnosis are reflective of the difficulty in diagnosing this condition. One relatively straightforward list of research criteria for possible PD includes [46]:

1. Evidence of disease progression.
2. Occurrence of at least two of the three cardinal features of Parkinsonism (rigidity, tremor, bradykynesia)
3. Occurrence of at least two of the following:
    a. Marked response to *L*-dopa (functional improvement or dyskinesia)
    b. Asymmetry of signs
    c. Asymmetry at onset
4. Absence of clinical features of alternative diagnosis
5. Absence of aetiology known to cause similar features

Other diagnostic guidelines incorporate requirements pertaining to disease duration, and more specifically regarding tremor and response to dopaminergic agonists [47]

1.5.4. Management

There is no cure for PD, yet prescriptions, surgery and multidisciplinary management can give easing from the manifestations. The principle groups of medications helpful for treating motor indications are levodopa (generally joined with a dopa decarboxylase inhibitor or COMT inhibitor), dopamine agonists and MAO-B inhibitors [48]. The stage of the ailment figures out which gathering is generally valuable. Two stages are normally recognized- an initial stage in which a single person with PD has effectively created some handicap for which he needs pharmacological medicine, then a second stage in which an individual creates motor confusions identified with levodopa usage [48]. Treatment in the initial stage goes for an ideal trade off between great manifestation control and reactions coming about because of change of dopaminergic capacity. The medication of levodopa (or *L*-DOPA) may be deferred by utilizing different solutions, for example, MAO-B inhibitors and dopamine agonists, for postponing the onset of dyskinesias [48]. In the second stage the point is to decrease side effects while controlling variances of the reaction to drug. Sudden withdrawals from prescription or abuse must be managed [48]. When solutions are insufficient to control manifestations, surgery and deep brain stimulation might be of use [49]. In the last stages of the malady, palliative consideration is given to enhance quality of life [50].

1.5.4.1. Levodopa

Levodopa has been the most generally utilized medication in excess of 30 years [48]. *L*-DOPA is changed over into dopamine in the dopaminergic neurons by dopa decarboxylase [48]. Since motor indications are created by an absence of dopamine in the substantia nigra, the organization of *L*-DOPA briefly lessens the motor symptoms [48]. Only 5–10% of *L*-DOPA crosses the blood–brain boundary. The rest is regularly metabolized to dopamine somewhere else, bringing about a mixture of reactions including vomiting, dyskinesias and joint stiffness [48]. Carbidopa and benserazide are fringe dopa decarboxylase inhibitors [48], which help to keep the digestion system of *L*-DOPA in check before it achieves the dopaminergic neurons, along these lines diminishing symptoms and expanding bioavailability. They are by and large given as mix arrangements with levodopa [48]. Existing arrangements are carbidopa/levodopa (co-careldopa) and benserazide/levodopa (co-beneldopa).

1.5.4.2. Catechol-O-methyl-transferase (COMT) inhibitors

Tolcapone restrains the COMT enzyme, which debases dopamine, in this way delaying the impacts of levodopa [48]. It has been utilized to supplement levodopa; nonetheless, its handiness is restricted by conceivable symptoms, for example, liver damage [48]. A likewise compelling drug, entacapone, has not been demonstrated to cause noteworthy adjustments of liver function [48]. Licensed arrangements of entacapone hold entacapone alone or in consolidation with carbidopa and levodopa [48].

1.5.4.3. Dopamine agonists

A few dopamine agonists that tie to dopaminergic post-synaptic receptors in the brain have comparable impacts to levodopa [48]. These were initially utilized for people encountering on-off variances and dyskinesias as a reciprocal help to levodopa; they are currently primarily utilized on their own as an initial treatment for engine manifestations with the point of deferring engine complications [48, 51]. Dopamine agonists incorporate bromocriptine, pergolide, pramipexole, ropinirole, piribedil, cabergoline, apomorphine and lisuride [48].

Dopamine agonists produce critical, in spite of the fact that typically mellow, reactions including laziness, mental trips, a sleeping disorder, sickness and constipation [48]. Sometimes symptoms seem even at an insignificant clinically viable measurement, heading the doctor to hunt down an alternate drug [48]. Compared with levodopa, dopamine agonists may postpone motor complexities but they are less successful at controlling symptoms [48].

1.5.4.4. Monoamine oxidase B (MAO-B) inhibitors

MAO-B inhibitors (selegiline and rasagiline) build the level of dopamine in the basal ganglia by hindering its metabolism. They hinder MAO-B which breaks down dopamine emitted by the dopaminergic neurons. The decrease in MAO-B action brings about expanded *L*-DOPA in the striatum [48]. Like dopamine agonists, MAO-B inhibitors utilized as monotherapy enhance motor indications and postponement of the requirement for levodopa in right on time illness, however create more unfavorable impacts and are less powerful than levodopa [48].

1.5.4.5. Other drugs

Other drugs, for example, amantadine and anticholinergics may be helpful as medication of motor manifestations. But then again, the quality proof is required, so they are not first line treatments [48]. Notwithstanding motor side effects, PD is joined by a differing extent of manifestations. Various drugs have been utilized to treat some of these problems [52]. Examples are the utilization of clozapine for psychosis, cholinesterase inhibitors for dementia, and modafinil for daytime sleepiness [52, 53]. A 2010 meta-investigation found that non-steroidal calming medications (separated from acetaminophen and headache medicine), have been connected with at any rate a 15 percent decrease of occurrence of the advancement of PD [54].