Dopamine Receptor Agonists for Protection and Repair in Parkinson's Disease

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Abstract: Dopamine agonists have been usually used as adjunctive therapy for the cure of Parkinson's disease. It is generally believed that treatment with these drugs is symptomatic rather than curative and it does not stop or delay the progression of neuronal degeneration. However, several dopamine agonists of the D2-receptor family have recently been shown to possess neuroprotective properties in different *in vitro* and *in vivo* experimental Parkinson's disease models. Here we summarize some recent molecular evidences underlining the wide pharmacological spectrum of dopamine agonists currently used for treating Parkinson's disease patients. In particular, the mechanism of action of different dopamine agonists does not always appear to be restricted to the stimulation of selective dopamine receptor subtypes since at least some of these drugs are endowed with antioxidant, antiapoptotic or neurotrophic properties. These neuroprotective activities are molecule-specific and may contribute to the clinical efficacy of these drugs for the treatment of chronic and progressive neurodegenerative diseases in which oxidative injury and/or protein misfolding and aggregation exert a primary role.

Keywords: Free radicals, oxidative stress, Parkinson's disease, Alzheimer's disease, beta amyloid, neurodegeneration, fibrils, neurotrophic factors, neurogenesis.

1. INTRODUCTION

Parkinson's disease (PD), Parkinson's disease with dementia (PDD), Lewy bodies dementia (DLB) and Alzheimer's disease (AD) are a heterogeneous group of neurodegenerative disorders characterized by gradually progressive, selective loss of anatomically or functionally related neuronal systems. Although it is not clear whether or not these diseases represent part of the same disease spectrum, they share common clinical phenomenology and pathological substrate but differ in spatial and temporal evolution of the disease process. Age appears to have a significant, if not dominant, effect in modifying disease expression within this spectrum. Despite this heterogeneity, neuronal cell loss is perhaps the most important common theme in these diseases. Thus, the development of disease-modifying therapeutic strategies that combine both symptomatic and neuroprotective effects is definitely required for effective management of these diseases.

PD is the most common neurodegenerative movement disorder. Approximately 2% of the population older than 65 years suffers from this slowly progressive neurodegenerative disease. More than 90% of PD cases are sporadic. The primary cause of the disorder is the progressive loss of the pigmentated DAergic neurons in the substantia nigra *pars compacta* (SNpc) accompanied by the appearance of intracytoplasmic inclusions known as Lewy bodies. Lewy bodies contain aggregated alpha synuclein. Interestingly, patients with abnormalities in the Parkin gene have a recessive, early onset Parkinsonism and do not have Lewy bodies. In contrast patients with dominant forms of hereditary PD and patients with idiopathic PD do have Lewy bodies [1]. As the disorder progresses, neurodegenerative changes and Lewy bodies are seen in other parts of the brain, including the cranial nerve nuclei, the nucleus basalis, locus coeruleus, and specific nuclei of the hypothalamus.

Many patients with PD develop dementia. In addition there is a type of dementia known as LBD in which Lewy bodies are found not only in the areas which contain neuromelanin, like the SN, but in numerous other areas including temporal lobe and cingulate gyrus. In the latter locations they are not as well demarcated or as distinct as the typical Lewy bodies. However, like all Lewy bodies, they can be stained with antibodies against ubiquitin [1, 2]. This makes them much easier to be detected and this test should be performed in any case of dementia where histology has ruled out AD and in which typical Lewy bodies have been recognized in SN or locus coeruleus. The relationship between PDD and LBD is a matter of some controversies. Moreover, cases that appear to have both AD and LBD have been reported and in these cases the burden of senile plaques and neurofibrillary tangles is less than that normally seen with dementia of the pure Alzheimer type. Amyloid plaques in sporadic LBD and AD show an identical biochemistry and progression pattern, suggesting a metabolic defect of amyloid precursor protein as a risk factor for synucleinopathy.

To date, the etiopathogenesis of nigral DAergic neuron loss in PD is unknown. However, the presence of ongoing oxidative stress as the result of inefficacious antioxidant defence mechanisms and generation of radical oxygen species (ROS) in the SNpc of the parkinsonian brain are considered to be important pathogenic mechanisms [3-5]. It should be noted that part of these free radicals are inevitably produced by DA metabolism in the brain either by enzymes through the action of monoamine oxidase-B or by auto-oxidation [6]. Other sources of increased radical production

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may be endogenous neurotoxins occurring in the brain like tetrahydroisoquinolines or exogenously administered neurotoxins like the widely used herbicide paraquat which have similar neurochemical properties like the well-known neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [7-9]. Moreover, it has been suggested that PD could be associated with excitotoxicity and apoptosis [10]. Therefore, an effective anti-parkinson therapy should not only alleviate the disease associated symptoms, but should also interfere with the progressive DAergic death in the SN.

Levodopa combined with a peripheral DOPA-decarboxylase inhibitor and a COMT inhibitor is considered the therapy of choice for PD. Levodopa is nearly always effective, but has a high incidence of adverse effects with long term use, including response fluctuations (on/off phenomena) and dyskinesias. DAergic agonists, acting directly at the receptor level, would be able to decrease the incidence of these motor complications. In the last 2 years, three new DA agonists have been launched, including ropinirole, pramipexole and cabergoline. These new agonists have been added as therapeutical options to well-established drugs, like pergolide, bromocriptine or talipexole [4, 5].

Here we review recent data underlining the potential pleiotrophic effects of some of the DA agonists currently used for the treatment of PD. The wide and molecule-specific pharmacological spectrum of these drugs may participate in restoring the impaired DA function by stimulating DA D2 receptors as well as in preventing neurodegenerative processes by additional pharmacological properties.

NOVEL PHARMACOLOGICAL PROPERTIES OF DA AGONISTS

The rationale for using DA agonists in the treatment of motor disturbances present in PD stands on the capability of these drugs to restore the striatal DAergic imput that is impaired in PD. We argue that, besides these pharmacological effects, DA agonists may inhibit intracellular death pathways and/or stimulate neuron regeneration. These effects may be a consequence of (specific) DA receptor stimulation or be completely independent from them. These additional pharmacological effects may also involve sites of action distal from the nigro-striatal areas and contribute to slow down the progression of the neurodegenerative process. We have selected four potential pharmacological effects that alone or in combination may enlarge the pharmacological spectrum of different DA agonists.

Antioxidant Activity

In progressive neurodegenerative diseases, such as PD, modification of the rate of disease progression is currently a highly debated topic. Increased oxidative stress is thought to be involved in nigral cell death, that is characteristic of PD. This oxidative stress may be further exacerbated by levodopa therapy. These mechanisms have been proven *in vitro* and in animal models, but their relevance in humans remains speculative [4, 5]. Based on these considerations, the emerging therapeutic strategies for PD advocate early use of DA agonists in the treatment of PD. A number of recent well-controlled studies have proven the efficacy of DA agonists used as monotherapy. Moreover, as predicted by animal studies, on the long term, DAergic agonists induce significantly less motor complications than levodopa.

It is generally believed that treatment with DAergic drugs is symptomatic rather than curative and it does not stop or delay the progression of neuronal degeneration. However, several DA agonists of the D2-receptor family (including D2 and D3 subtypes) have recently been shown to possess neuroprotective properties in different *in vitro* and *in vivo* experimental PD models. Most DA agonists have demonstrated protective properties in cell culture against a range of toxins, including DA, 6-hydroxydopamine (6-OH-DA), 1-methyl-4-phenylpyridinium (MPP^+) and hydroxy peroxide [4, 5, 11]. DA agonists also protect against toxin action *in vivo*, as shown in rodents receiving intrastriatal injection of 6-OH-DA or MPP^+. As reported in details in the following chapters, at cellular level, independent groups have demonstrated decreased free radical production and an amelioration of DA neuronal loss following DA agonist treatment.

Stimulation of Neurogenesis

The neurotransmitter and its receptors appear early during ontogenesis and affect cell proliferation in the embryonic germinal zones [12, 13]. Interestingly, the regulation of neural stem cells by DA persists in the restricted brain areas where neurogenesis occurs in adulthood, particularly in the subventricular zone (SVZ) within the lateral wall of the lateral ventricles and in the subgranular zone of the hippocampus. Ongoing adult neurogenesis is currently believed to be an important form of neural plasticity, enabling organisms to adapt to environmental changes and possibly influencing learning and memory throughout life. On the other hand promotion of adult neurogenesis may offer a potential approach for replacing neurons or neuritic networks that degenerate or lose function during aging or in neuropathological settings, including PD. This therapeuthic strategy is particularly intriguing since in post-mortem brains of individuals with PD the numbers of neural precursor cells in neurogenic regions are dramatically reduced [14]. In a very elegant study Hoglinger and coauthors [14] provided experimental evidences that the highly proliferative precursors cells located in the adult murine subependymal zone lining the lateral ventricle receive dopaminergic afferents. This innervation appears functionally relevant. Transient and bilateral dopaminergic denervation by administration of MPTP resulted in transient and bilateral decrease in the subependymal zone proliferation. Additionally, ablation of mesencephalic dopaminergic neurons of adult rats by stereotaxic injection of 6-OHDA into the nigrostriatal pathway resulted in the unilateral dopaminergic denervation of the subependymal zone as well as in the marked reduction in the number of proliferating precursors. Conversely, cell proliferation was restored by L-DOPA chronic infusion. Similar results were obtained in the hippocampal neurogenic region, which has been shown to receive a dopaminergic from the ventral tegmental area. More recently, the existence of a topographically organized projection from the SNpc to the SVZ was also demonstrated in primates [15], with the anteromedial SNpc projecting to the anteroventral SVZ and the posterolateral SNpc to the posterodorsal SVZ.

The effects on precursor cell proliferation elicited by dopamine are likely to be mediated by D2-like receptors, since D2 and D3 receptors are expressed on neural stem/progenitors cells. As recently summarized by Joyce and Millan [16], different D3 receptor-preferring agonists augment mitogenesis in the SVZ. In particular, Van Kapen and associates demonstrated induction of neurogenesis leading to the regeneration of DAergic pathways, suggesting that this effect may participate in the restoration of DAergic nigrostriatal pathway and locomotor activity in a rat model of PD [17, 18]. However, species-specific differences of D3 receptors in regulating neurogenesis have been reported [19, 20]. A two-fold induction of cell proliferation in the SVZ and rostral migratory stream of the adult rats was demonstrated following icv administration of the dopamine D(3) receptor agonist, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) for 2 weeks [17]. The same drug had no effect on mice [20, 21]. More robust are the data on the involvement of D2 receptors in regulating neurogenesis, based on both the use of D2-null mice and D2 agonists/ antagonists [22].

Whether or not reduced neurogenesis may be a consequence of or rather contribute to the pathophysiology of PD certainly deserves further investigation since these studies may have significant implications for the future design of novel therapeutic strategies.

Anti-Fibrillary Activity

Very recent data suggest anti-fibrillary effects of DA agonists. The aggregation of β-amyloid peptide (Aβ) and alpha-synuclein in the brain has been implicated as a critical step in the development of AD and LBD, respectively. Thus, in addition to antioxidant strategies, increasing evidence points to the possibility of achieving neuroprotection by DA agonists through inhibition of fibril formation [23]. DAergic agents were indeed found to dose-dependently inhibit generation of, as well as destabilize preformed, β-amyloid fibrils [24]. Using fluorescence spectroscopy with thioflavin S, electron microscopy, and atomic force microscopy, the effects of selegiline, DA, pergolide, bromocriptine, and trihexyphenidyl on alpha-synuclein fibrils formation have been recently studied. All molecules, except for trihexyphenidyl, dose-dependently inhibited the formation of alpha-synuclein fibrils. Moreover, these molecules dose-dependently destabilized preformed alpha-synuclein fibrils [25]. The anti-fibrillary actions elicited by the DA agonists appear to be independent of DA receptor stimulation and detectable in cell-free systems.

Stimulation of Neurotrophic Factors

One of the most convincing evidence for the role of neurotrophic factors in PD is the very recent study of Elsworth *et al.* [26]. They found that implantation of adeno-associated virus type 2 encoding glial derived neurotrophic factor (AAV2-GDNF) in the normal monkey caudate nucleus induced overexpression of GDNF that persisted for at least 6 months after injection. In a 6-month within-animal con-trolled study, AAV2-GDNF enhanced the survival of fetal dopamine neurons by 4-fold, and increased the outgrowth of grafted fetal dopamine neurons by almost 3-fold in the caudate nucleus of MPTP-treated monkeys, compared with control grafts in the controlateral caudate nucleus. GDNF is a potent neurotrophic factor that is crucial for development, survival, and outgrowth of DA neurons [27, 28]. GDNF is highly expressed in the developing rat striatum, yet its concentration is relatively low in the adult brain [29-31]. Several studies in the 6-OH-DA lesioned striatum of rats have demonstrated improvements in survival and outgrowth of grafted fetal DA neurons when SNC injections of GDNF have been administered, or when GDNF overexpressing cells have been co-grafted to the striatum [32-38].

Other than GDNF, brain-derived neurotrophic factor (BDNF) is one of the major trophic factors for DAergic neurons [39-41]. Transplantation of modified fibroblasts or astrocytes that express BDNF into either the striatum or the midbrain attenuates 6-OH-DA-induced loss of nigrostriatal neurons [42-43]. Also, BDNF can modulate dopaminergic neurotransmission in nigrostriatal neurons, as shown by elevated rotational behavior and increased turnover of dopamine in the striatum [39]. BDNF can promote functional recovery from 6-OHDA lesions following expression in striatal cells from an AAV vector [44].

These studies support the relevance of neurotrophic factors for a neurorestorative effect expected in the treatment of PD. As reported in the following chapters, some DA agonists have been found to stimulate either BDNF or GDNF in experimental cell cultures. These pharmacological effects may be useful to improve treatment for PD.

2. EXPERIMENTAL EVIDENCE FOR NEURO-RESCUE ACTIVITY

Apomorphine

Apomorphine, a non-ergoline DA receptor agonist, is a short-acting and non-selective DA D1/D2 receptor agonist used for treating PD since many decades. Subcutaneous intermittent injections or continuous infusions of apomorphine have been proposed for the management of sudden, unexpected and refractory levodopa-induced “off” states in fluctuating PD [45].

The original evidence for neuroprotective and antioxidant effects of apomorphine, and other DA receptor agonists, is from Youdim’s group [46]. Rationale for their pivotal studies was the relevance of ROS generation in the neurotoxicity associated with PD [3, 6]. Since compounds with a catechol structure have metal chelating properties and can act as reducing agents [47], it was attractive that apomorphine may inhibit metal-catalyzed free radical processes and act as a free radical scavenger. Studies on the effect of apomorphine on lipid peroxidation and protein carbonyl formation after ascorbate/iron-induced free radical formation in rat brain mitochondrial fractions clearly demonstrated the antioxidant properties of apomorphine in brain tissue [46, 48].

It has later been shown that apomorphine exhibited neuroprotection against DA depletion in 6-OH-DA lesioned-rats [49] and MPTP-treated mice [50]. Furthermore, continuous subcutaneous infusion of apomorphine was found to rescue striatal DAergic terminals and increase the tyrosine hydroxylase and DA-transporter immunoreactivity against toxicity induced by MPTP in mice and enhanced the number

of tyrosine hydroxylase-positive cells in the ventral tegmental area in partially 6-OH-DA-lesioned rats [51, 52]. It has also been observed that apomorphine increased the survival of cultured mesencephalic DAergic cells [53] suggesting trophic effects of apomorphine either *in vivo* or *in vitro*. The neuroprotective effect of apomorphine was further supported by the result of different pharmacological properties including antioxidant activity, potent iron chelating action, inhibition of lipid peroxidation, induction of neurotrophic factors and anti-inflammatory effects [46, 50, 54-56].

Recently, specific brain gene expression changes have been reported in the chronic MPTP model in the late stage of degeneration, employing cDNA expression array, which indicating a domino cascade of events involved in neuronal cell death [57]. Specifically, the authors detected alterations in the expression of genes implicated in oxidative-stress, inflam-matory processes, signal transduction and glutamate toxicity. Induction of these pro-toxic genes appeared to be prevented in mice pretreated with apomorphine.

Bromocryptine and Quinpirole

Bromocryptine is the first DA receptor agonist that has been approved for anti-parkinsonian therapy since 1974 [58]. It was first used as adjunctive therapy to levodopa in patients experiencing motor fluctuations and later it was recommended as monotherapy in the early stage of the disease [59]. From a pharmacological point of view, bromo-cryptine was the first DA receptor agonist endowed with D2 receptor specificity to be described. Bromocryptine has been shown to protect mice and DAergic cells against 6-OH-DA and MPTP, and levodopa-induced cell loss, respectively; it also attenuated DA depletion in mouse striatum in response to methamphetamine [60-62]. The neuroprotective effect of bromocryptine was dependent on both its action as a D2 receptor agonist and its antioxidant capacity. In this context, it has been reported that bromocryptine is able to scavenge hydroxyl and superoxide radicals *in vitro* [61, 63] and to inhibit hydroxyl radical formation and lipid peroxidation *in vivo* [61].

Glutamate, the main excitatory neurotransmitter in the mammalian brain, contributes to the neuronal death associated with neurodegenerative diseases, such as AD and PD, and with ischemia. Bromocryptine has been shown to exert a protective effect against glutamate-induced cytotoxicity in primary cultures of rat cortical or mesencephalic neurons [64] The neuroprotective effect was mediated via D2 receptors, because it was attenuated by domperidone, a D2 DAergic receptor antagonist. Another DA D2 agonist, quinpirole, also protected cells against glutamate toxicity. In particular, the D2 agonists protected cells from calcium influx, nitric oxide, and peroxynitrite toxicity, which are thought to be the mediators of glutamate toxicity. The phosphatidylinositol 3 kinase (PI3K) inhibitor LY294002 inhibited this neuroprotective effect of bromocriptine. Furthermore, Akt protein kinase, which is an effector of PI3K, was activated by bromocriptine, and the antiapoptotic protein Bcl-2 was up-regulated by bromocriptine treatment. These results suggested that D2 DAergic receptor activation plays an important role in neuroprotection against glutamate cytotoxicity and that the up-regulation of Bcl-2 expression via the PI3K cascade is, at least partially, involved in this effect.

Recently, Marvanova and Nichols [65] investigated mechanisms of PD in Caenorhabditis elegans (C. elegans). In general, striking and profound similarities underlie the basic cellular and molecular processes between the worm and humans. The use of C. elegans over traditional mammalian-based systems holds the promise of an enhanced rate of discovery with lower associated costs. In particular, they utilized C. elegans to screen a variety of compounds, including specific DA receptor agonists, such as bromocriptine and quinpirole, to identify those that protect against 6-OH-DA-induced DAergic toxicity. The two DA D2 receptor agonists were found to protect against 6-OH-DA toxicity in a dose-dependent manner. Surprisingly, these protective effects appear to involve receptor-independent mechanisms.

Pergolide

Among DAergic agonists, the synthetic ergoline derivate pergolide mesylate has recently been indicated in monotherapy as an efficacious and well-tolerated first-line treatment in patients with early stage PD. However, its clinical use has been limited, since it was recently shown to be associated with the development of restrictive valvular heart disease [66-68].

The neuroprotective effect of pergolide has been observed both *in vivo* and *in vitro*. Chronic administration of pergolide preserved the integrity of nigrostriatal neurons in the aging rat brain [69] and protected neurons against reduction of striatal DA and its metabolites after 6-OH-DA injection in mice [70]. Using cell culture models, pergolide has been shown to promote the survival of DAergic neurons, to exhibit partial protection against MPP^+ toxicity and to increase ^{3}H-DA uptake by cultured cells after levodopa treatment [71]. The neuroprotective effect of pergolide has been shown to be mediated by free radical scavenging activity particularly hydroxyl radicals and nitric oxide and by decreasing phospholipid peroxidation [72, 73] suppressing apoptotic pathways by inhibiting of NF-κB nuclear translocation [74] and stabilizing the mitochondrial function [71]. In clinical trials pergolide was shown to reduce the long-term decline of striatal fluorodopa uptake compared to levodopa treatment, however, without reaching statistical significance [75].

Data obtained in our laboratory demonstrated that pergolide protected SH-SY5Y neuroblastoma cells from cell death induced by H_2O_2 [76]. Neuroprotection was elicited when pergolide was added to the culture medium either simultaneously or before H_2O_2 treatment. Neuroprotection by pergolide was not prevented by preincubation of the cells with phenoxybenzamine, spiperone, SCH23390 or (-)-sulpiride, suggesting that drug effects were not mediated by DA receptor stimulation. Moreover, the neuroprotective effect of pergolide was specific for H_2O_2, since doxorubicin or cis-platinum- induced cell death was not affected by the presence of the DA agonist drug in the culture medium.

Treatment of SH-SY5Y neuroblastoma cells with pergolide significantly prevented glutathione depletion [76]. Since oxidative stress in SH-SY5Y neural cells is known to

activate NF-κB pathway we tested the hypothesis that pergolide may interfere with NF-κB transcriptional activity [77, 78]. We showed that exposure of human SH-SY5Y neuronal cell line to H_2O_2 results in NF-κB nuclear translocation, activation of p53 and p53-target genes, including p21, Gadd45, MSH2 and Bax, and apoptosis. The cell-permeable SN50 peptide, which is known to block NF-κB/Rel nuclear translocation, prevented both H_2O_2-induced p53 expression and apoptosis. These results suggested that, in this particular cell model, NF-κB activation is involved in the H_2O_2-induced apoptosis. Similarly to the SN50 peptide, pergolide inhibited NF-κB/Rel nuclear translocation in SH-SY5Y neuronal cell line exposed to H_2O_2; this effect resulted in the lack of p53 transcriptional activation and prevention of apoptosis [74].

Ropinirole

Ropinirole is a non-ergoline DA receptor agonist that exhibits a high affinity for the D2 and D3 receptor subtypes but little or no affinity for the D1 receptor [79, 80]. Symptomatically, it was reported that ropinirole was as effective as bromocriptine in reducing motor complications and decreasing levodopa dose without increasing adverse events including dyskinesia [81]. Also, ropinirole monotherapy was effective in treating resting tremor in early PD [82], in reducing periodic leg movements and in improving sleep efficiency in patients with restless leg syndrome [79, 83]. These positive effects of ropinirole in PD are believed to be due to stimulation of the post-synaptic DA D2-type receptor [80]. In experimental models of PD, it has been found that ropinirole reversed the motor and behavioral deficits induced by MPTP in marmosets [80] and showed neuroprotective effect against 6-OH-DA in mice [84]. Activation of glutathione and glutathion-regulating enzymes such as glutathione peroxidase, glutathione reductase and glutathione transferase as well as activation of catalase and superoxide dismutase were principal neuroprotective mechanisms mediated by ropinirole [85].

In clinical trials ropinirole reduced the long-term decline of striatal fluorodopa uptake compared to levodopa therapy indicating a preserving effect on DAergic nerve endings [75, 86]. Previous *in vitro* studies showed that ropinirole can promote the differentiation and survival of DAergic neurons, and it can upregulate the expression and secretion of GDNF and BDNF [87]. Considering the potential effects of ropinirole on neuroprotection, it has been suggested that ropinirole may have an anti-apoptotic effect by interfering with MAP kinase and caspase-dependent pathway.

Recent studies showed that ropinirole has neuroprotective effect against rotenone-induced apoptosis in both SH-SY5Y cells and primary mesencephalic cultures [88]. Exposure to rotenone significantly activated p-JNK, p-P38 and p-c-Jun in SH-SY5Y cells. Activation of JNK and P38 was responsible for the inhibition of the anti-apoptotic protein Bcl-2, and the induction of phosphorylation of c-Jun, a nuclear transcription factor and a known target of JNK, which further promotes the release of cytochrome c from the mitochondria to the cytoplasm and it leads to caspase-9 activation [89-92]. Pretreatment with ropinirole inhibits p-JNK, p-P38 and p-c-Jun expression, indicating that ropinirole may act at early stage of apoptosis. These effects appear to be mediated by neurotrophic factors GDNF and BDNF, since both of them were found to increase in the primary mesencephalic cultures after treatment with ropinirole and pramipexole [87, 93]. The anti-apoptotic effect of ropinirole can be suppressed by D2 and D3 receptor antagonists sulpiride and nafadotride. Nafadotride exhibited greater effect in blocking ropinirole mediated anti-apoptosis, suggesting that D3 receptor may play a significant role.

Pramipexole

Pramipexole, a non-ergoline DA agonist, has been success-fully applied to the treatment of PD. Similarly to ropinirole, pramipexole exhibits a high affinity for the D2 and D3 receptor subtypes but little or no affinity for the D1 receptor. The neuroprotective effects elicited by this drug have directly and/or indirectly been associated with antioxidant effects, mitochondrial stabilization or induction of the antiapoptotic Bcl-2 family. In particular, Le *et al*. [94] reported that pramipexole protected DAergic MES 23.5 cell line against DA, 6-OH-DA and hydrogen peroxide-induced cytotoxicity possibly through antioxidant effects, and such neuroprotection was independent from DA receptor stimulation not being prevented by selective D2 or D3 antagonists. Similar results were obtained by Fujta *et al*., [95] and Uberti *et al.* [96], who demonstrated that pramipexole inhibits the generation of H_2O_2-induced reactive oxygen species in PC12 cells and SH-SY5Y neuroblastoma cells, respectively.

Because of its antioxidant properties, pramipexole has been proposed to be effective against other pathologies, beside PD, where oxidative stress is the main mechanism implicated in pathophysiology of the diseases. In fact, oxidative abnormalities have been identified both in familiar and sporadic amyotrophic lateral sclerosis (ALS). The treatment of patients affected by ALS with pramipexole reduced the systemic production of oxygen radicals, as demonstrated by measuring the levels of 2,3-dihydroxybenzoic acid (2,3-DHBA) in the blood, suggesting that pramipexole may interrupt free radical production in ALS [97]

In search for an appropriate cell model for studying neuroprotection, Presgraves *et al.* [98] characterized differentiation conditions of the SH-SY5Y neuroblastoma cell line for phenotypic markers of DAergic cells, Cells were differentiated with retinoic acid (RA), 12-O-tetradecanoylphorbol-13-acetate (TPA), and RA followed by TPA (RA/TPA). Interestingly, RA/TPA differentiated cells exhibited 3-fold and 6-fold higher levels, respectively, of DA D2 and D3 receptors than undifferentiated or RA-differentiated cells. Pretreatment with pramipexole was protective against MPP^+ in the RA/TPA differentiated cells but not in undifferentiated or RA differentiated cells. The neuroprotective effect of pramipexole was concentration-dependent and DA D2/D3 receptor dependent. In contrast, protection by pramipexole against DA was not DA receptor dependent. This model system provides unique information about DA receptor dependent and independent mechanisms of neuroprotection.

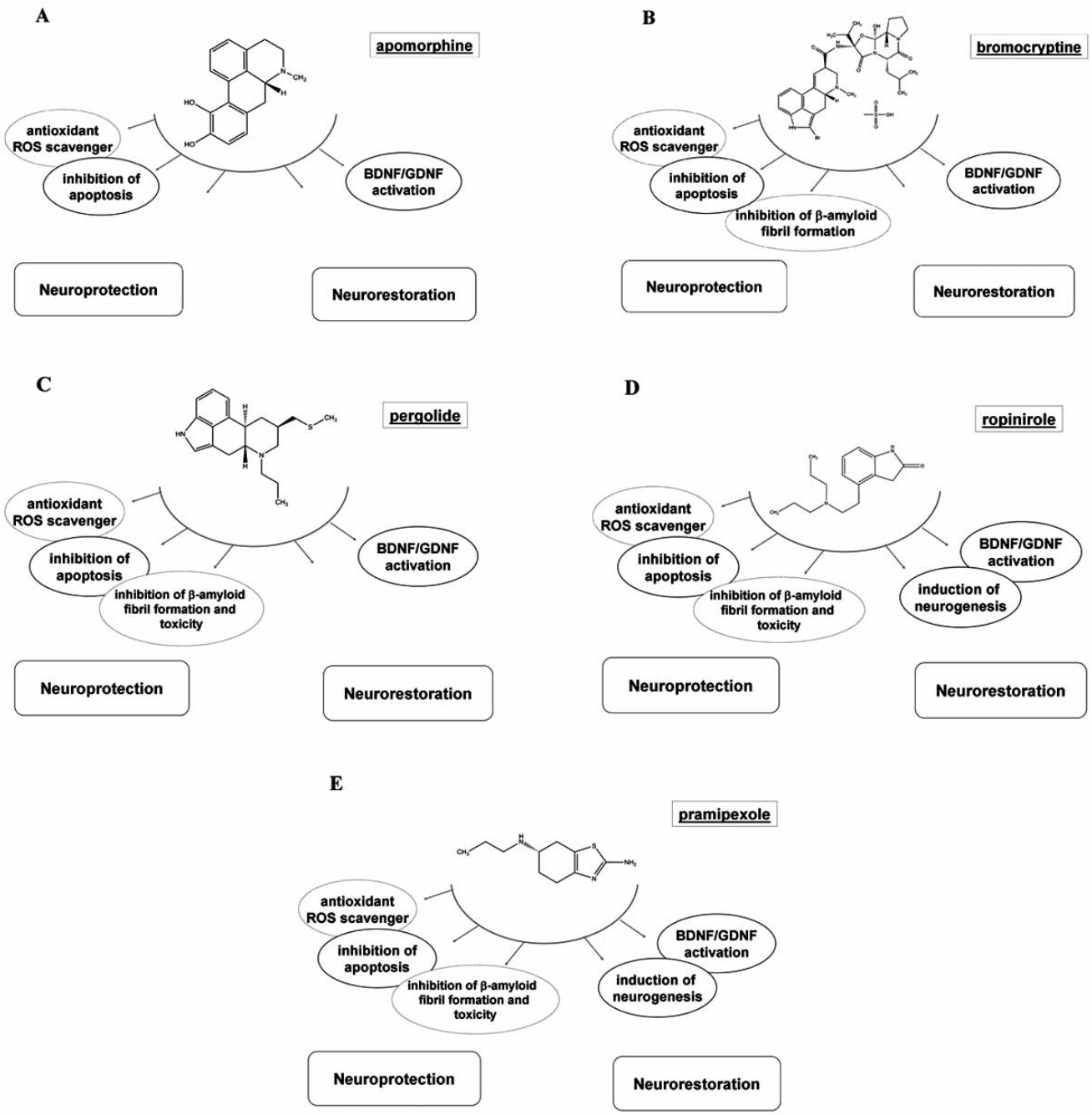

Fig. (1). Schematic representation of the pharmacological properties of apomorphine (A), bromocryptine (B), pergolide (C), ropinirole (D), and pramipexole (E) that are responsible for putative neuroprotection and neurorestorative effects.

An additional mechanism underlying protection by D2/D3 receptor agonists has been suggested to involve neurotrophic factors. To verify this hypothesis, the D3 receptor preferring agonists S32504 [(+)-*trans*-3,4,4*a*,5,6,10*b*-hexahydro-9-carbamoyl-4-propyl-2*H*-naphth[1,2-*b*]-1,4-oxazine] and pramipexole were utilized in a terminally differentiated neuroblastoma SH-SY5Y cell line exhibiting a DAergic phenotype [99]. The cytotoxic effects of MPP^+ were stereospecifically antagonized by S32504 and by pramipexole, but not by their inactive stereoisomers, R(+) pramipexole and S32601, respectively. Neuroprotective effects afforded by S32504 and pramipexole were specifically antagonized by the selective D3 antagonists S33084, U99194A, and SB269652, and by the D2/D3 antagonist raclopride. The preferential D2 receptor antagonist LY741626 was ineffective as the D1 antagonist SCH23390.

BDNF potently protected against MPP^+-induced neurotoxicity. An antibody directed against BDNF concentration-dependently blocked both the neuroprotective effects of BDNF and those of pramipexole and S32504 against MPP^+. The protection afforded by BDNF was blocked by the PI3K-AKT pathway inhibitor LY249002. Neuroprotective effects of pramipexole and S32504 against MPP(+) toxicity appear to be mediated by D3 receptors stimulation. Their actions also reflect downstream recruitment of BDNF via a PI3K-AKT pathway.

An increasing amount of evidences suggest a central role of oxidative stress also in AD pathogenesis [100, 101]. Furthermore, many findings link free radical formation with excessive Aβ deposition [102-105]. Aβ is a 39-43 amino acid long peptide derived from the amyloidogenic processing of the larger amyloid precursor protein [106, 107]. The basic hypothesis in AD has been that enzymatic cleavage of the amyloid precursor protein generates the release of monomers of Aβ, that undergo conformational changes concomitant with self assembly into oligomers and then fibrils. During the fibril formation process, the peptides acquire neurodegenerative properties. The ability of toxic Aβ peptides to induce protein oxidation and inhibit the activity of oxidation-sensitive enzymes is consistent with the hypothesis that Aβ can act like a pro-oxidant agent [103, 108]. We recently studied the role of free radical in the neurotoxic events caused by different Aβ aggregation states, and investigated the neuroprotective effects of pramipexole in neuronal death induced by unaggregated, oligomeric and fibrillary Aβ species [96]. Increasing evidences are now available suggesting that protofibrillar aggregates of Aβ recognized as diffuse plaques by neuropathological examination, are indeed the most toxic Aβ species. Oligomers of Aβ, rather than monomers or large fibrils, may form pores in the cell membrane, allowing influx of ions, that disrupt neuronal signalling and initiate cell death cascade [109, 110]. These data strongly support the hypothesis that each of the Aβ aggregation state possesses different biological and pathological functions We challenged the neuronal SH-SY5Y cell line with $A\beta_{1-42}$ peptide in different states of aggregation. Contrary to unaggregated peptide, oligomers and fibrils caused generation of ROS and this effect was inhibited by pramipexole in a DA receptor independent manner. The interaction of pramipexole with Aβ activity is further supported by the data showing that pramipexole prevented the induction of caspase 3 activated by $A\beta_{25-35}$ [111].

3. PERSPECTIVES

Neuroimaging Studies in PD Patients

To evaluate the possibility that DA agonists may indeed prevent DAergic cell loss a number of recent clinical studies compared rates of DA neuron degeneration after treatment with ropinirole, pramipexole, or levodopa by means of neuroimaging techniques [86, 112]. These trials included neuroimaging substudies to analyze for possible neuroprotection by DA agonists. The CALM-PD study used β-CIT SPECT scanning techniques at baseline and month 23 in 82 patients [113, 114]. The mean change per year of CIT uptake was similar for both treatment groups. In the ropinirole study 28 ropinirole-treated patients and 9 levodopa-treated patients were evaluated. A trend was seen toward preservation of DAergic function in the striatum in the ropinirole group, especially in those patients who had the disease for less than two years. A similar trend was found in the pergolide trial using PET studies [115].

Radiotracer imaging of the nigrostriatal DAergic system is a widely used but controversial biomarker in PD. Biomarkers used to study disease biology rely on evidence that they are measuring relevant biologic processes. The four radiotracers used as biomarkers in PD, namely [^{18}F] fluorodopa PET, (+)-[^{11}C]dihydrotetrabenazine PET, [^{123}I]β-CIT SPECT, and [^{18}F]fluorodeoxyglucose PET, fulfil this criterion, although they do not measure the number or density of DAergic neurons. Biomarkers used as diagnostic tests, prognostic tools, or surrogate endpoints must not only have biologic relevance but also a strong linkage to the clinical outcome of interest. No radiotracers fulfil these criteria, and current evidence does not support the use of imaging as a diagnostic tool in clinical practice or as a surrogate endpoint in clinical trials [116, 117].

Dopamine Agonists and Dementia

Prevalence of dementia in community-based PD cohorts is between 20% and 40%. The development of dementia in PD is associated with a twofold increased mortality, reduced quality of life, increased caregiver distress, and nursing home placement. Dementia also limits the drug treatment of extrapyramidal motor impairments, because L-DOPA may exacerbate psychotic features and confusion [2, 118, 119].

From a neuropathological and neurochemical point of view, cholinergic deficits are more marked in PD patients with dementia compared to those without, as evidenced by greater neuronal loss in the nucleus basalis of Meynert. Furthermore, by using a ligand of vesicular acetylcholine transporter, PDD cases demonstrate extensive cortical binding decrease similar to AD. In PDD, extrastriatal DAergic and particularly cholinergic deficits appear to play a central role in mediating dementia. Management of dementia in PD represents one of the major therapeutic challenges. Still, it is not yet well established if cognitive impairment is part of PD or the clinical manifestation of an independent, concomitant neurodegenerative disease [118-119]. Despite this unsolved question, we propose that DAergic drugs may be useful in delaying the progression of the neurodegenerative processes. In fact, independently from the etiopatogenesis of the specific neurodegenerative disease that is responsible for the cognitive impairment, they share common deleterious processes, i.e. neuronal loss and protein aggregation. In this regard, it should be noted that DA agonists were found to be active in preventing Aβ aggregation and ROS formation [22-25, 96].

A large body of recent data indicates considerable heterogeneity between different neurodegenerative diseases, including AD, PD, PDD, and LBD. Many of the clinical syndromes and pathologies overlap, and, due to molecular interaction between various proteins, many phenotypes show mixed neuropathologies. Only few are featured by one single principal pathology with no or only minimal additional lesions. Diagnosis is thus hampered by the onset of the main neurological symptom and it is often unchanged by the occurrence of delayed clinical manifestations. In this regard,

several clinical and epidemiological studies linking vascular factors to the pathogenesis of AD strongly argue that the large majority of sporadic AD patients show a so-called "mixed dementia" characterized by neurodegenerative and vascular lesions typical of both AD and vascular dementia [120-122].

4. CONCLUSION

The mechanism of action of different DA agonists currently used for the treatment of PD patients does not appear to be entirely restricted to the restoration of the decreased striatal DA input through the stimulation of selective DA receptor subtypes. These drugs are endowed with intrinsic and peculiar antioxidant, antiapoptotic, neurotrophic and anti-fibril formation effects. These activities are molecule-specific and may represent additional pharmacological properties contributing to their clinical efficacy in PD.

In conclusion, since the independent and synergistic multi-sites of action, we propose selective DA agonist drugs as coadiuvants in the treatment of chronic and progressive neurodegenerative diseases in which oxidative injury and/or protein misfolding and aggregation exert a primary role. However, the molecular evidence suggesting additional pharmacological properties of the DAergic drugs deserve further clinical studies to determine if these drugs can indeed slow down the neurodegenerative disease progression.

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ABBREVIATIONS

6-OH-DA	=	6-Hydroxydopamine
AAV2	=	Adeno-associated virus type 2
Aβ	=	β-Amyloid peptide
BDNF	=	Brain derived neurotrophic factor
COMT	=	Cathecol-O-methyl transferase
DA	=	Dopamine
DOPA	=	3,4-Dihydroxyphenylalanine
GDNF	=	Glial derived neurothophic factor
LBD	=	Lewy bodies dementia
MPP	=	1-Methyl-4-phenylpyridinium
MPTP	=	1-Methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine
PD	=	Parkinson's disease
PDD	=	Parkinson's disease with dementia
PI3K	=	Phosphatidylinositol 3 kinase
RA	=	Retinoic acid
ROS	=	Radical oxygen species
SNpc	=	Substantia nigra *pars compacta*
SVZ	=	Subventricular zone
TPA	=	12-O-Tetradecanoyl-phorbol-13-acetate
β-CIT	=	[^{123}I]2β-Carbomethoxy-3β–(4–iodophenil) tropane

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