Natural Products and Derivatives Affecting Neurotransmission Relevant to Alzheimer's and Parkinson's Disease

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Abstract

The two major neurodegenerative diseases Alzheimer's disease (AD) and Parkinson's disease (PD) are characterised by low levels in the brain of the neurotransmitters acetylcholine (ACh) and dopamine (DA), respectively. Clinical treatment of these two conditions is palliative and relies, in most cases, on improving stimulation at the relevant receptors by either increasing levels of the endogenous neurotransmitter or by the use of substances which have a similar agonist response. Natural products continue to provide useful drugs in their own right but also provide templates for the development of other compounds. The major advances in the treatment of AD have been the use of acetylcholinesterase inhibitors such as galantamine, huperzine A, physostigmine and its derivatives to increase the levels of ACh rather than the use of cholinergic compounds, although compounds with nicotinic properties have attracted some interest. In contrast, the treatment of PD has relied on the elevation of DA levels by use of L-DOPA, its precursor,

and by the administration of dopaminergic agonists, especially the ergot alkaloid derivatives. The use of inhibitors of enzymes that cause breakdown of DA is an avenue which is being explored. As well as the major natural products of clinical interest, the paper discusses the chemistry, activity and usage of the constituents of plants used in traditional medicine for the treatment of diseases presenting symptoms similar to those characteristic for Alzheimer's or Parkinson's disease.

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Introduction

Neurodegenerative disease is a generic term applied to a variety of conditions arising from a chronic breakdown and deterioration of the neurons, particularly those of the central nervous system (CNS). In addition, these neurons may accumulate aggregated proteins which cause dysfunction. Alzheimer's disease (AD) and Parkinson's disease or parkinsonism (PD) are the two best-known diseases of this type and will be the main diseases associated with this review. However, some specialists would classify multiple sclerosis and spongiform encephalopathies as neurodegenerative diseases. The latter have received publicity in recent years due to the link between Creutzfeldt-Jacob disease (CJD) in humans and 'mad cow disease'. Spongiform encephalopathies have been shown to be

caused by prions, proteinaceous particles synthesized within the cell which replicate and accumulate in cells and so alter protein structure and therefore function but, as yet, there are no drugs available to treat these conditions.

Most commonly, neurodegenerative disease manifests in elderly people and, in advanced industrialised and post-industrialised societies, where life expectancy is long, this group of conditions is a major cause of morbidity and of death, as well as imposing severe strains on the social welfare systems. To illustrate this it is reported that, in the USA, AD now affects at least 5 million people and in the UK in 2001 it affected 750,000 people and was the fourth most common cause of death [1]. However, it is increasingly becoming recognized by the World Health Organisation as a global problem [2]. The common symptoms of neurodegenerative diseases, such as loss of memory and tremor, have been recognised as a feature of increasing age for a long time and are acknowledged in many traditional medical systems. However, it is only comparatively recently that, as distinctive diseases, they have been recognized and received much attention from mainstream medicine. This is most likely due to the fact that, in the past, short life expectancy precluded many surviving to an age where neurodegeneration was likely to affect a significant part of the population.

The etiology of neurodegenerative diseases is still largely unknown but, especially for AD and PD, postmortem studies have shown clear links between the disease and a deficiency of neurotransmitters in parts of the brain. Thus, in AD there is a chronic shortage of acetylcholine (ACh) 1 and in PD a deficit of dopamine (DA) 2. Until very recently, the only clinical treatment for both of these conditions was the reversal of these deficiencies by elevation of the levels of the transmitter by agonists, or by inhibition of enzymes involved in their removal from the immediate locality of the synapse. These approaches are discussed more thoroughly below. A variety of natural products have been shown to play roles which would have the desired effects and some of these, or their derivatives, have been brought into clinical use. This paper gives an overview of such compounds, as well as others, with interesting activity which have not been developed as drugs or are still undergoing clinical trials. It should not be forgotten that there are also many traditional medicinal plants with a reputation of alleviating or preventing symptoms of neurodegeneration and that these are used as crude extracts and mixtures [3, 4]. In some cases, such extracts have been shown to relieve neurodegenerative symptoms in animal models or display relevant *in vitro* activity, but both the modes of action and identity of any compounds responsible have not been fully determined.

Increasingly attention is being paid to the excitatory amino acid neurotransmitters and relevant receptors which are present in the CNS, e.g. the N-methyl-D-aspartate (NMDA) receptor. The use of these in designing new drugs or of explaining the use of traditional medicinal plant extracts is in its infancy and no significant findings have been made of natural products which either bind to the receptors or affect levels of the transmitters.

Alzheimer's Disease

AD is the major disease of a group which is characterised by loss of cognitive function leading to dementia. AD is estimated to account for between 50 and 60% of dementia cases in persons over 65 years of age [5] and is a progressive, neurodegenerative disease that primarily affects the elderly population. It is a major public health concern in developed countries because of the strains imposed on carers and financial resources by the increasing numbers of sufferers. The main symptoms associated with AD involve a decline in cognitive dysfunction, primarily memory loss [6, 7] and in the later stages of the disease language deficits, depression, agitation, mood disturbances and psychosis are often seen [8].

Although AD, as a defined medical condition, has only existed for about 100 years, age-related loss of memory and cognitive decline has been documented for thousands of years in human history. In common with many other conditions, ancient writings which describe the symptoms also suggest remedies, based usually on plant extracts. Thus, Ashwagandha (*Withania somnifera*) is mentioned in ancient Sanskrit writings from India as a 'medharasayan' or promoter of learning and memory retrieval whilst in the sixteenth century Gerard's Herbal from the UK, sage *Salvia officinalis*, is described as being 'good for the memory'. In recent years, the value of a few of these has been demonstrated scientifically and some of the compounds mentioned below have been isolated from traditional medicinal plants.

Postmortem studies have shown that AD is characterized by low amounts of the enzyme choline acetyl transferase (ChAT) and enzyme abnormalities which would produce levels of the neurotransmitter ACh 1 (fig. 1) [9, 10] and there also appears to be a depletion of nicotinic function. Attention deficit in AD is reversed with nicotine 3, which is reported to upregulate nicotinic receptors and to increase ACh release, so enhancing cholinergic neurotransmission [11, 12].

Fig. 1. Neurotransmitters of the CNS relevant to neurodegenerative disease and the agonist nicotine.

ACh is certainly associated with cognitive function, since situations where it is blocked from acting on the cholinergic receptors by drugs such as hyoscine (scopolamine) 4 (fig. 2), which is a muscarinic antagonist, result in severe cognitive impairment in the patient. It is still unclear if the low levels of ACh in the CNS are cause or effect as far as AD is concerned, but the repletion of levels has been exploited therapeutically with some success in the last 15 years in the symptomatic relief of AD. The synthetic compound tacrine was the first drug introduced into the clinic and it increased the levels of ACh by inhibition of acetylcholinesterase (AChE), the enzyme responsible for fast breakdown of ACh after its release from the nerve ending. This inhibition results in ACh having a longer half-life and therefore increasing in concentration at the synapse. Tacrine was the first of several AChE inhibitors which have come into clinical use, but it is no longer used since the more recent introductions, generally named second generation inhibitors, are safer and have longer-lasting effects. These recent introductions include at least two based on natural products. It should be stressed that AChE inhibitors only alleviate some of the cognitive symptoms of the disease for a time and ultimately do not arrest the cognitive decline of the patient.

Another approach which has been proposed is the employment of cholinergic and, to some extent, nicotinic agonists, but this has not proved to be as useful therapeutically as the inhibition of cholinesterase.

More recently prevention of glutamate-mediated neurotoxicity has been a therapeutic target in AD. The N-methyl-D-aspartate (NMDA) receptor antagonist, memantine, has been introduced in some countries for clinical use in AD patients. It should be noted that other types of activity, besides increase of neurotransmitter levels, are

Fig. 2. Scopolamine, a muscarinic receptor antagonist.

also being explored as leads for chemotherapeutic treatment of AD. Activities being explored include antioxidant, anti-inflammatory and inhibition of β -amyloid synthesis, but these are not covered to any extent in this paper.

AD: The Use of Cholinergic Compounds

The rationale underlying the use of cholinergic compounds is that they are agonists of the nicotinic cholinergic receptor and so compensate for the low levels of ACh. The binding of ACh to the receptor is shown diagrammatically in figure 3. It can be seen that the important features required in a molecule are an amine which becomes positively charged at the pH of the immediate environment and so binds to an aspartate in the receptor, a portion of the molecule able to form hydrogen bonds with the asparagine domain of the receptor and small groups able to bind to hydrophobic sites near the aspartate region. In addition, the receptor lies in a pocket which allows only small molecules to enter.

Although these compounds have been suggested as valuable agents in treating AD, because they also appear to inhibit fibrillary tangle and amyloid production, success has been limited as far as clinical studies are concerned, although results in animals were initially promising [13]. Two major alkaloidal natural products are known to have this effect, arecoline 5 and pilocarpine 6 (fig. 4). It can be seen that these molecules are small and that they incorporate at least two of the criteria noted above for binding to the receptor.

Arecoline is the major alkaloid present in areca or betel nut, the fruit of the palm tree *Areca catechu* L. (Areaceae), which is extensively used as a masticatory throughout the Indian subcontinent and other parts of southeast Asia. It is estimated that 500 million people regularly chew betel nut (often referred to as 'pan' in India) in a

form which is usually shredded, mixed with lime and wrapped in a leaf of *Piper betel* L. Excessive salivation occurs, which is a direct result of its cholinergic activity, which also gives the mild CNS stimulation for which the product is principally used. There was some interest in arecoline as a treatment for AD since it showed improvement in memory tests in rats [14]. Arecoline has been shown to bind to the M2 muscarinic receptors but not the nicotinic receptors [15]. A small clinical study showed that, when arecoline was given continually by intravenous infusion in AD patients, it enhanced verbal memory [16]. Derivatives of arecoline have been synthesized in order to improve selectivity for cortical muscarinic ACh receptors and two examples are Lu 25-109 and talsaclidine 8 which are M₁ receptor agonists. Although Lu 25-109 showed encouraging results in vitro [17], it failed to improve cognition when tested clinically in patients with mild to moderate AD [18]. Talsaclidine has been shown to increase cholinomimetic central activation in animals and humans without some of the side effects seen with AChE inhibitor therapy, but cognitive function was not significantly improved [19]. Tests on rhesus monkeys did however show some improvement in memory-related tasks but at doses which gave unacceptable side effects [20].

Pilocarpine 6 is one of a series of related alkaloids found in species of the South American plant genus *Pilocarpus*, known commonly as Jaborandi leaf, which was used in traditional medicine since it induced sweating and urination, features which were perceived as being useful for eliminating toxins from the body. The molecular structure of pilocarpine 6 bears similarities to ACh since the positively charged N atom and the lactone binding to the serine are about the same distance apart. Chewing the leaf causes copious salivation as well as other typical features of cholinergic stimulation such as contraction of the pupils. Pilocarpine has been shown to enhance memory performance in aged rats [21], but no studies on its application in humans for the treatment of AD have been reported and it appears to have been discarded as a potential therapeutic lead. This is possibly due to its poor pharmacokinetic profile as it cannot pass through the blood-brain barrier, as well as its undesirable side effects.

AD: The Use of Cholinesterase Inhibitors – Alkaloids

To avoid the undesirable effects of excess cholinergic stimulation, ACh is rapidly hydrolysed after release at the synapse by an enzyme named acetylcholinesterase AChE.

Fig. 3. Binding of acetylcholine to nicotinic receptor.

Fig. 4. Cholinergic compounds.

A similar enzyme, butylcholinesterase BuChE, also occurs. If the cholinesterase is inhibited, the ACh does not hydrolyse so quickly, and levels of ACh rise. The 'classic' cholinesterase inhibitor is the alkaloid physostigmine 9, also called eserine. This was isolated from the Calabar Bean, the seeds of *Physostigma venenosum* Balf., in the nineteenth century in studies stimulated by the use of the seeds as an ordeal poison in what is now southeastern Nigeria.

The toxic effects of calabar bean extract were found to be due to excessive cholinergic stimulation resulting in increased salivation, nausea, bradycardia, muscle cramps and respiratory failure, as well as CNS effects such as agitation. The cholinergic excess was found to be caused by inhibition of the rapid breakdown of acetylcholine by physostigmine.

Fig. 5. Interaction between acetylcholine and the acetylcholinesterase active site.

In recent years, the structure of AChE has been determined and the mode of binding for AChE inhibitors has also been elucidated. A variety of AChEs exist according to the source species, but they vary only in small details and all contain the active site at the base of a deep cleft in the enzyme.

Figure 5 illustrates the particular amino acid residues which are considered to be the most important in the binding process and from this knowledge, structure-activity relationships of AChE inhibitors can be understood at the intermolecular level. The important regions of an inhibitor appear to be a positively charged nitrogen, which binds to an aspartate residue, and a region, separated by a lipophilic area from the positive charge, which can form a hydrogen bond with a tyrosine or serine residue. A positively charged nitrogen is common in many alkaloids at body pH and it is not surprising that many of the most powerful AChE inhibitors are alkaloids. In recent years, however, a number of natural products, mentioned below, which do not contain any nitrogen, have been found to be AChE inhibitors. This raises questions about their binding characteristics to AChE since the key role played by a positively-charged moiety of the molecule bonding with the anionic aspartate residue, can no longer account for the activity. The interactions between the enzyme and such non-alkaloidal compounds have generally not yet been investigated.

The cholinesterase inhibitors (fig. 6) cause overstimulation of a number of functions in many animal species and exert a considerable toxic effect even at quite low

Fig. 6. Acetylcholinesterase inhibitors.

doses. This has been exploited in the area of insecticides, and the insecticide carbaryl was developed by making synthetic analogues of physostigmine. In therapeutics, cholinesterase inhibition had, until recently, a somewhat limited application in only ophthalmology and the treatment of myasthenia gravis. However, the realisation that early symptoms of AD could be improved by the use of cholinesterase inhibitors awakened renewed interest and, since physostigmine was known to cross the blood-brain barrier, several *in vivo* studies were conducted which showed that it reduced symptoms of ACh deficiency in the CNS. Physostigmine was reported to protect mice against cognitive impairment caused by oxygen deficit and it improved learning in rats [22]. Clinical studies showed significant cognitive benefits in both normal and AD patients [23], but it had a short half-life, which has prevented its application clinically in AD patients, since this would require multiple daily dosing.

As well as inhibiting AChE, physostigmine also inhibits butyrylcholinesterase (BuChE), another enzyme found in the CNS, so adverse effects associated with BuChE inhibition, such as gastrointestinal disturbance, may also occur with physostigmine. However, BuChE has recently been implicated in the aetiology and progression of AD [24], so inhibition of BuChE may therefore prove to be beneficial in treating AD and physostigmine and other BuChE inhibitors such as rivastigmine may have clinical efficacy superior to AChE-selective inhibitors.

To improve its pharmacokinetic profile, there is a considerable history of the synthesis of analogues of physostigmine. These have been applied to the treatment of myasthenia gravis, neostigmine 10 being the most widely used drug for this disease. Neostigmine is a quaternary amine and this feature severely impairs its ability to cross the blood-brain barrier and so be of value in treating AD. Rivastigmine 11 was produced with the express purpose of engineering a better pharmacokinetic profile for usefulness in AD and it inhibits the G_1 form of AChE in the cortex and hippocampus, brain areas involved in cognition [25], and it has been shown to improve cognition in AD patients [26, 27]. Clinical studies have borne out the usefulness of rivastigmine (Exelon®) in mild to moderate AD and it has been licensed as a treatment for symptomatic relief of AD since 2000.

Galantamine 12 (sometimes referred to as galanthamine) is found in members of the Amaryllidaceae, e.g. the Chinese medicinal herb *Lycoris radiata* Herb. and the European *Galanthus nivalis* L. and *Narcissus* spp. [28]. The ethnopharmacological uses of plants containing this compound are not very clear, but a full report of the history of the development of galantamine from *Galanthus nivalis* has been recently published [29]. Its cholinesterase inhibitory properties were first exploited in Bulgaria in the mid-twentieth century for the treatment of polio victims, but it only came into prominence as a treatment for AD in the last decade of the twentieth century.

Galantamine has been licensed in Europe for AD treatment since 2001. Multi-centre randomised clinical trials showed that it was well tolerated and significantly improved cognitive function when administered to AD patients [30–32]. The cognitive benefits appear to be sustained for at least 3 years, a much longer time than for other drugs of this type [33]. Galantamine is well absorbed when given orally and is also more selective for AChE than BuChE [34]. It also stimulates nicotinic receptors indirectly by its allosteric potentiation of ACh [35], and so may also enhance cholinergic function and memory (see below). This effect suggests that galantamine may

have therapeutic advantages over other AChE inhibitors and its value in vascular dementia as well as AD is recognised in recent studies [36]. Several other alkaloids with AChE inhibitory activity have been recently reported from members of the Amaryllidaceae, but they have not been subjected to vigorous pharmacological and clinical testing. Several other alkaloids isolated from Iberian *Narcissus* species have been tested for cholinesterase activity [37]. A study of two *Crinum* species used in Nigerian traditional medicine to help ailing memory resulted in the isolation of four alkaloids of which the most active was hamayne 13, although its IC_{50} value of 250 μM was three orders of magnitude weaker than physostigmine, so it is unlikely that it would be present in sufficient quantity to have a strong therapeutic effect [38].

Whilst physostigmine analogues and galantamine have entered the pharmacopoeia in the Western world, another natural cholinesterase inhibitor, huperzine A 14 has been introduced in China for treating AD. Huperzine A is one of the alkaloids found in the clubmoss *Huperzia serrata* Thunb. (Lycopodiaceae) which is used in various formulae in traditional Chinese medicine (TCM) to alleviate problems of memory loss, promote circulation and for fever and inflammation [39]. Huperzine A is related to the quinolizidine alkaloids and it reversibly inhibits AChE in vitro and in vivo [40, 41].

Huperzine A has been shown to improve memory in cognitively impaired rats [42] and in gerbils following ischaemia [43]. These observations suggest that huperzine A has clinical potential in cerebrovascular disorders, as well as in AD. Indeed, since it has also been shown to be neuroprotective, AChE inhibition may not be the only explanation for the clinical effects observed. Huperzine A has been shown to be neuroprotective against β -amyloid peptide fragment 25–53 and free radical-induced cytotoxicity [44] and to attenuate apoptosis by inhibiting the mitochondria-caspase pathway [45]. In a multi-centre, double-blind trial, huperzine A significantly improved memory and behaviour in AD patients, and was reported to be more selective for AChE than BuChE and was less toxic than the synthetic AChE inhibitors donepezil and tacrine [36]. A randomised, placebo-controlled study on over 200 patients, 100 of whom were given 400 μg huperzine A daily for 12 weeks and showed significantly higher scores for improvement compared with the placebo group in the various scorings used [46]. Recent progress on many aspects of huperzine A has been reviewed [47].

Other alkaloids have shown AChE activity and most of these have been isolated from plants used in traditional medicine (fig. 7). *Coptis chinensis* Franch. (Ranunculaceae)

Fig. 7. Minor alkaloidal cholinesterase inhibitors.

has been used in TCM for several conditions including age-related cognitive and memory decline. Some alkaloids found in this species, such as berberine 15, coptisine 16 and palmatine 17, are reported to also be anti-ChE [48, 49]. *Coptis chinensis* extract improved a scopolamine-induced learning and memory deficit in rats [50] and this is likely to be due to the alkaloids present raising ACh levels.

Berberine 15 has been shown to be selectively active against AChE compared with BuChE [51] and it has been shown to improve scopolamine-induced amnesia in rats [52]. Rutaecarpine 18 is the major alkaloid found in *Evo-dia rutaecarpa* (Juss.) Benth. (Rutaceae), the unripe fruit of which is used in TCM for cardiogenic, restorative and analgesic effects. Pharmacological activities relevant to AD have been identified with the extract and with rutaecarpine. Rutaecarpine inhibited COX-2 activity in vitro, and was anti-inflammatory in vivo [53]. Dehydroevodiamine 19, another alkaloid found in the same plant,

inhibited AChE in vitro, and reversed scopolamine-induced memory impairment in rats [54]. It also increased cerebral blood flow in vivo in cats, a property which would supplement its usefulness in AD [55]. Solanidine 20 and related compounds and their glycosides are steroidal alkaloids produced in green parts of the genus *Solanum*, which includes the potato. The toxic properties of these alkaloids have been known for a long time and have been shown to be due to AChE inhibition, with characteristic signs of cholinergic excess such as sweating, palpitations and CNS disturbances including hallucinations [56]. Reports of *Solanum* species being used to treat AD or related conditions in traditional medicine do not exist and the alkaloids have not been investigated for any usefulness clinically, presumably because of their toxicity.

Although it is comparatively easy to explain the fact that some alkaloids inhibit AChE because of their molecular features, it is less easy to correlate chemical structure and activity for some other phytochemical types for which AChE inhibition has recently been reported.

AD: The Use of Cholinesterase Inhibitors – Terpenoids and Other Types of Compound

Terpenoids comprise a very large group of natural products and comprise two or more branched 5 carbon units, formed from a common precursor named mevalonic acid. Skeletons consisting of multiplets of 2, 3, 4 or 6 of these linked together in many different ways are found in a variety of mostly cyclic compounds named monoterpenes (10 carbons in the skeleton), sesquiterpenes (15 carbons), diterpenes (20 carbons) and triterpenoids (30 carbons), respectively (fig. 8). These compounds tend to be lipophilic, so they are able to cross the blood-brain barrier, and the monoterpenes, and some of the sesquiterpenes, are volatile, and so effects could occur through inhalation. These compounds are responsible for the strong odours and flavours of many herbs, spices and traditional medicines. Many such molecules are increasingly recognized as having a variety of roles in living organisms, including signalling between members of the same species, thus comprising part of the group of compounds known as pheromones, and protective or attractant roles in flowering plants against herbivores and pollinators respectively. An effect on CNS activity by the volatile substances in perfumes and other odoriferous materials has attracted interest in recent years and one of the first findings that monoterpenes had AChE inhibitory effects was made only in the mid 1990s in studies investigating

historical records that monoterpene-containing plants were 'good for the memory' [57].

One such group of plants was the various European species of *Salvia*, commonly known as sage. An ethanolic extract and oil of *S. officinalis* L. (Labiatae) and *S. lavandulaefolia* Vahl. (Labiatae) were investigated for anti-ChE activity and it was found that all gave inhibition of AChE at quite low concentrations [57].

The cholinesterase inhibition shown by the *S. lavandulaefolia* oil was shown to be partly due to the cyclic monoterpenes 1,8-cineole 21 and α -pinene 22, which were shown to inhibit AChE in vitro, with some contribution from other constituents, perhaps by acting synergistically [58, 59]. However, the monoterpenes were considerably less active, by a factor of at least 10^3 , than the alkaloidal AChE inhibitors such as physostigmine 9 [58].

Since the effects of the oil were better than those of individual monoterpenes, further in vivo and clinical studies, described below, were carried out on the essential oils, which consist of a mixture of monoterpenes, rather than isolated compounds. Oral administration of *S. lavandulaefolia* essential oil to rats decreased striatal AChE activity in both the striatum and the hippocampus compared to the control rats. Thus, it appeared that constituents of the *S. lavandulaefolia* oil, or their metabolites, reach the brain and inhibit AChE in select brain areas, consistent with evidence of inhibition of the brain enzyme in vitro [60].

Clinical studies on human volunteers and even patients with AD have been reported in recent years. The effect of sage in twenty participants using a placebo-controlled, double-blind, balanced, cross-over design showed significant effects on cognition associated with the lowest dose of *Salvia*, including improvements in both immediate and delayed word recall scores [61]. A small trial with 11 patients showing mild to moderate symptoms of AD showed that oral administration of the essential oil of *S. lavandulaefolia* significantly improved cognitive function in one of the three different methods of assessment used [62]. Another, more thorough, trial in Iran on 48 patients with similar symptoms of AD showed that those treated with an extract of *S. officinalis* gave significantly better values in measurements of cognitive function [63].

Melissa officinalis L. (Labiatae) leaf is another species that contains monoterpenes in its essential oil. It has been used as a medicinal plant for more than 2000 years and has a reputation of for promoting long life and for restoring memory [64]. Recent studies have focused on the reputed cognitive effects of *M. officinalis*. A recent randomised, placebo-controlled, double-blind, balanced

Fig. 8. Terpenoid cholinesterase inhibitors.

crossover study showed an improvement in cognitive performance and mood in 20 healthy young participants, following treatment with dried leaf of *M. officinalis* shown by previous in vitro tests to be cholinergically active [65]. In another study, an extract of *M. officinalis* was administered to patients with mild to moderate AD for 4 months and gave a significantly better outcome on cognitive function than placebo [66]. Although the constituents present have not been investigated, numerous monoterpenes have been identified in the essential oil of *M. officinalis*, including citral 23 (a mixture of the isomers geranial and neral) and it is known that these are weak inhibitors of AChE [67].

The root of another species of *Salvia*, *S. miltiorrhiza* Bung. (Labiatae) is extensively used in TCM to stabilise the heart and calm nerves [48]. *S. miltiorrhiza* has been the subject of thorough investigation, and consequently numerous pharmacological activities that may be relevant in CNS disorders, including AD, have been identified. *S. miltiorrhiza* has been employed for the treatment of cerebral vascular disease, and there are several studies to

Fig. 9. Steroidal derivatives with anticholinesterase inhibitory activity.

investigate possible mechanisms for the protective effect of *S. miltiorrhiza* against cerebral ischaemia.

There is evidence that *S. miltiorrhiza* root extract may protect neurons from ischaemia and attenuate dysfunction of neuropeptides of importance in neurodegenerative disease [68]. An inhibitory effect on AChE has been recently demonstrated and has been shown to be due to the diterpenes present known as tanshinones [69]. Dihydrotanshinone 24 was shown to be the most active ($IC_{50} = 1.0 \mu M$) with cryptotanshinone 25 ($IC_{50} = 7.0 \mu M$) also showing activity. A feature which appears to be necessary for activity is the saturated bond in the furan ring of the molecules.

Screening of 139 different Indian medicinal plants and spices for AChE inhibitory activity led to *Origanum majorana* L. (Labiatae) showing the highest activity. The active component was identified as the triterpene ursolic acid 26, a fairly common compound, which exhibited an IC_{50} value of $7.5 nM$, less than an order of magnitude weaker than that of the positive control tacrine [70]. This result is of interest in view of the widespread occurrence of ursolic acid and may account for the traditional use of several plant species for memory improvement and AD-related conditions.

The withanolides are a group of compounds related to the steroids which are found in some genera of the Solanaceae, notably *Withania somnifera* (L.) Dun. The root of this plant is one of the most highly regarded herbs in Ayurvedic medicine where it is known as 'ashwagandha' and has a history of use for almost 4,000 years. It is classed among the rejuvenative tonics known as 'Rasayanas' and several groups have described the cognitive enhancing potential of extracts of the roots in experimental animals. Some individual compounds have also been investigated and the sitoindosides IX 27 and X (fig. 9) have been shown to augment learning acquisition and memory in both young and old rats [71]. The mechanisms to explain this effect are unclear, but may involve modulation of cholinergic neurotransmission. An extract containing the sitoindosides VII–X and withaferin A 28 was administered to mice and effects on the neurotransmitter systems in the brain were observed. The results from this study showed that the extract enhanced AChE activity in the lateral septum and globus pallidus areas of the brain and also enhanced muscarinic M_1 receptor binding in cortical regions, but it did not affect γ -aminobutyric acid (GABA) $_A$, benzodiazepine receptor binding, nor NMDA or amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptor subtypes [72].

The extract containing the sitoindosides VII–X and withaferin A also reversed the reduction in cholinergic markers (e.g. ACh, choline acetyltransferase; ChAT) in rats [73]. These activities could explain the reputed cognition enhancing effects of *W. somnifera* root because of preferential action on cholinergic neurotransmission in the cortical and basal forebrain, brain areas involved in cognitive function. Based on this information, it could be speculated that the sitoindosides and withaferin A could have potential in AD therapy, but more studies need to be carried out before they can be used with any degree of confidence in being able to achieve this or whether the crude extract might produce a better effect.

In addition to their cholinergic activity, the glycowithanolides showed anxiolytic and anti-depressant activities in rats [74], which may be applicable in the symptomatic treatment of AD.

W. somnifera root and some constituents are also reported to have anti-oxidant properties which may also be relevant in AD therapy [75]. Anti-inflammatory effects of the extract of roots have been demonstrated in rats [76] and the extract has also been shown to reduce levels of the pro-inflammatory interleukins IL-1 and TNF- α , which are considered to be involved in senile plaque formation and neurodegeneration [77].

There are many types of phenolic compounds, but the only ones which have been shown to possess AChE inhibitory activity are neolignans from *Magnolia officinalis*. The root and stem bark of *Magnolia officinalis* Rehd. Et Wils. (Magnoliaceae) have been used in TCM to treat anxiety and nervous disturbances. *M. officinalis* contains the biphenolic lignans, honokiol 29 and magnolol 30 (fig. 10). Both lignans increased ChAT activity and inhibited AChE activity in vitro, and increased hippocampal ACh release in vivo [78]. Honokiol and magnolol show anxiolytic effects [79] and these appear to be due to their ability to potentiate GABAergic neurotransmission [80]. These two compounds also appear to have antioxidant anti-inflammatory and neuroprotective properties and such polyvalency in activity is of interest in their potential use in treating AD [81, 82].

AD: Use of Nicotinic Compounds

A link between smokers and a lower incidence of AD has been noted and this is thought to be associated with increased nicotine intake, although some recent reports present a contrary view in linking smoking with an increased incidence of AD [83, 84]. Nicotine 3 is reported to have cognition-enhancing effects and these may be due to nicotinic receptor stimulation but also protection against AD by other mechanisms such as inhibition of β -amyloid formation [85], inhibition of the neurotoxic effects of excitatory amino acids (e.g. glutamate) and enhancement of the effects of nerve growth factor (NGF) [86]. There are several other alkaloids which are nicotinic agonists at the cholinergic receptor [87] (fig. 11). Lobeline 31 from *Lobelia inflata* interacts with the nicotinic receptor [88] and could also be exploited to influence cholinergic function in AD. Other alkaloids such as sophoramine 32 and cytisine 33, found in members of the Leguminosae, have nicotinic actions. Cytisine is used as a pharmacological tool because of its strong binding affinity to nicotinic receptors, but it does not appear to have been developed for any pharmaceutical purposes, probably because of its toxicity.

Parkinson's Disease

Parkinson's disease is named after the English surgeon who first described a syndrome which he called the 'shaking palsy'. It affects a large number of people (about 20,000 in the UK) and is most commonly seen in older

Fig. 10. Neolignans with anticholinesterase inhibitory activity.

Fig. 11. Nicotinic alkaloids.

patients, although an estimated 5% of sufferers are under 40 years old [89]. Its characteristic feature is an increasing tremor in resting limbs and a rigidity, known as dyskinesia, particularly exhibited as a shuffling gait, but it is also associated with the degeneration of cognitive function and memory.

It is thought that oxidative stress in the substantia nigra plays a significant role in the loss of neurons which produce DA [90]. This results in the characteristic deficiency of DA in the substantia nigra seen in the brain of PD patients post mortem, the other characteristic histopathological observation being the presence of deposits called Lewy bodies in the surviving neurons.

The major therapeutic approach to PD has been to elevate the levels of DA by either inhibition of monoamine oxidase (MAO), which metabolises DA to less active compounds, or by increasing the concentration of the precursor of DA by administering *L*-hydroxyphenylalanine (*L*-DOPA) 34 (fig. 12). Another approach involves the use of compounds which are agonists on the DA receptors.

Fig. 12. Dopamine and related compounds.

PD: Use of Dopaminergic Agonists

The structure-activity relationships of compounds which are agonists at the DA receptor are generally accepted to be the two ortho OH groups on the aromatic ring which bind with serine residues 505 and 508, the aromatic ring enabling hydrophobic interactions with a phenylalanine at 617 and, in the terminal position of the ethyl amino group attached to the aromatic ring, a nitrogen with a positive charge which interacts electrostatically with an aspartate residue (fig. 13). It is noteworthy that the adrenergic receptor is very similar in some respects (fig. 14) and so it is possible that compounds with a structure favouring binding to one of these receptors, also have some effect on the other.

DA itself is quite unstable and cannot cross the blood-brain barrier. However, it can be formed within the brain by conversion of its precursor L-DOPA. This compound

Fig. 13. Interaction between dopamine and amino acid residues in the dopamine receptor.

Fig. 14. Interaction between adrenaline (epinephrine) and amino acid residues in the adrenergic receptor.

is now administered routinely to PD patients. It is found in commercially viable amounts in various species of bean, notably *Mucuna* spp., and this has been used as a commercial source, although the drug is now mainly obtained by synthesis. L-DOPA is often given together with another analogue of DA, carbidopa 35, which is not dopaminergic but which inhibits dopa-decarboxylase and so, by maintaining levels of L-DOPA in the blood, prolongs its activity. When L-DOPA is given over long periods of time, it is common for a sudden decline in sensitivity to occur from time to time and this is called the 'on-off' effect. This is probably due to a number of factors including depletion in the ability of the substantia nigra cells to store DA and desensitization of the receptors. Other dopaminergic agents, some of which are mentioned below, are often used as adjuvants to reverse the 'off' effect.

It is interesting that the powdered seeds of *Mucuna pruriens* L. have been used in Ayurvedic medicine for dis-

eases of the nervous system [91] and this product has shown to reduce adverse effects, such as the 'on-off' effect, in patients [92]. A commercial extract of *M. pruriens* HP-200 was shown to be twice as effective as the equivalent dose of L-DOPA in rats [93]. This may be due to the presence of other, more active, compounds. However, a later study showed that when the same preparation was given to rats over a 52-week period, it elevated DA levels in the cortex but not in the striatum nigrum, this calling into question whether the observed improvements in parkinsonian symptoms were due to the hypothesis originally proposed, i.e. that the L-DOPA in the *Mucuna* extract was converted to DA and reached the parts of the brain where a deficiency is associated with PD [93].

The transmitter dopamine DA is one of a group of compounds known as phenylpropylamines, which includes some important neurotransmitters such as adrenaline (epinephrine) 36 and noradrenaline (norepinephrine) 37. The phenylpropylamines also include several naturally-occurring compounds known as protoalkaloids, since their N atom is not part of a heterocyclic ring. Most protoalkaloids appear to have a greater adrenergic than dopaminergic effect, although it appears that they can stimulate both types of receptor. The major naturally-occurring compound of this type used therapeutically is ephedrine 38, obtained from some species of *Ephedra* (Ephedraceae), and these plants which have been used for many centuries in TCM. Ephedrine is used principally for its adrenergic sympathomimetic properties in drying up secretions, but it is well-known for its CNS-stimulant side effects, which may be due also to dopaminergic properties. In contrast, the synthetic phenylpropylamines known as amphetamines are primarily employed as CNS stimulants and there appears to be some association between their use and alleviation of the dyskinesia often associated with PD. A natural compound very similar in chemistry and pharmacology to the amphetamines is cathinone 39, a major active constituent of *Catha edulis* Forsk. (Celastraceae). The fresh young leaves of this plant are known as 'khat' and are used as a stimulant masticatory in Ethiopia, Yemen and surrounding countries and by the diaspora of those communities in Europe [94]. Cathinone has been shown to be present mainly in the young leaves only and, since there have been anecdotal reports of reduction in PD-like tremors induced by neuroleptic drugs in some regular chewers of khat, it might be that this is due to a similar effect of cathinone as that observed for amphetamines [95].

Probably the second most important group of compounds used for the treatment of PD are derivatives of the

Fig. 15. Ergot alkaloid derivatives used to treat parkinsonism.

ergot indole alkaloids (fig. 15). Ergot, *Claviceps purpurea* Tulane (Clavicipitaceae), is a fungus which infects the ears of cereal crops, notably rye *Secale cereale* L. Ergot has a long history of poisoning animals and humans who eat the cereal flour contaminated with ergot, but also has been exploited in traditional medicine in some parts of Europe to aid childbirth, since an extract causes contraction of the uterus towards the end of pregnancy and also constricts blood vessels, thus reducing bleeding which often occurs at birth. The effects of ergot on the CNS has also been well-documented and outbreaks of 'madness' involving hallucinations have been shown to correlate with times of heavy contamination of rye flour in the communities affected. The activity of ergot has been shown to be due to the alkaloids present such as ergotamine 40. All of the alkaloids contain the indole ring structure known as lysergic acid and similarities with the three neurotransmitters noradrenaline (norepinephrine), DA and serotonin can be seen in this (fig. 16). This probably explains the wide spectrum of activity of these alkaloids and so it has been found necessary to alter the chemical structure to produce compounds which more specifically bind to only one of the receptors. A large amount of derivatives of ergot alkaloids have been synthesized for differing therapeutic effects

Fig. 16. Ergot alkaloid skeleton showing parts similar to neurotransmitters.

Fig. 17. Other alkaloids with dopaminergic and monoamine oxidase inhibitory activity.

and one of these has been dopaminergic receptor stimulation for use in treating PD. Bromocriptine 41, pergolide 42, cabergoline 43 and lisuride 44 are examples of compounds which have been developed in this way and are now used clinically. The pharmacological differences between the compounds are not very great. All are D₂ dopamine receptor agonists, although pergolide also has agonist effects at D₁ and D₃ receptors. All four drugs are well-established in treatment and numerous reviews exist on their clinical efficacy and application [96, 97].

Bromocriptine and lisuride were the first of these drugs to be introduced and have comparatively short half-lives. Pergolide has a half-life of 8 h [98] and cabergolide of 68 h, making the latter especially useful for administration only once a day [99]. The ergot-derived drugs were originally used in advanced cases of PD, but there is increasing interest in their use in the first instance since, unlike L-DOPA, they do not need to be metabolized to the active compound and so are not affected by any degeneration in the dopaminergic terminals [100].

Another alkaloid with agonist activity is apomorphine 45, derived from the opium alkaloids (fig. 17). It acts on the D₁ and D₂ receptors, but, because it is a powerful emetic when given orally, its use is restricted to parenteral administration. It is most commonly given when patients are not exhibiting adverse effects to L-DOPA and as a diagnostic agent for dopaminergic responsiveness. The isoquinolinoid salsolinol 46 has been investigated extensively over the last 10 years. This compound is found in the cocoa bean, the seeds of *Theobroma cacao* L. (Sterculiaceae), and also in chocolate derived from this plant. The addictive properties of chocolate have been ascribed to the dopaminergic activity of this compound [101], but it also occurs as an endogenous catechol in the brain. Salsolinol has been shown to be dopaminergic at the D₂ receptors and also to have a protective effect on neurodegeneration [102]. The N-methyl derivative, which can be synthesized in the

brain, has a role in the pathogenesis of PD [103], and this complicates the usefulness of giving salsolinol as a protective substance to prevent or alleviate PD.

Mention should be made of another indole alkaloid, ibogaine 47, obtained from the African plant *Tabernanthe iboga* Baill. (Apocynaceae), the roots of which were used as a stimulant and, in larger doses, as a hallucinogen in central Africa. This compound has enjoyed much attention recently because of its claimed usefulness in combating addiction, particularly to cocaine, although the basis of its activity is not clear. Ibogaine was shown to release DA in isolated striatal tissue in mice [104], but it has also been shown to inhibit the release of catecholamines by blocking the nicotinic receptor [105] and to block NMDA receptors. These activities preclude its usefulness in treating neurodegenerative disease, but much remains to be done in the study of its antiaddictive potential.

The tropane alkaloids, especially hyoscyne (scopolamine) 4, have been used to treat PD since they antagonise cholinergic activity at the muscarinic receptors in the striatum and so increase DA activity. The naturally-occurring alkaloids, found in various genera of the Solanaceae such as *Atropa*, *Hyoscyamus*, *Datura* and *Duboisia*, are not much used for this purpose, but synthetic derivatives and analogues such as benztropine 48 are used. These also inhibit DA reuptake, so increasing the levels of DA and compensating for the DA deficiency associated with PD.

PD: Monoamine Oxidase Inhibitors

Monoamine oxidase (MAO) is a major enzyme responsible for the fast breakdown of DA and related compounds at the synapse. Inhibitors of this enzyme, known as MAOIs, cause a net increase in DA levels and, although their major therapeutic use has been as antidepressants, they have potential use in PD. This has not generally been realised because of the side effects associated with elevation of peripheral DA levels. The β -carboline alkaloids harmine 49 and harmaline 50 are found in several traditional medicine plant species, including *Banisteriopsis caapi* (Spruce ex Griseb.) Morton (Malpighiaceae), a liana used in Brazil as an ingredient in the hallucinogenic drink 'ayahuasca'. It is thought that the MAOI properties of the *B. caapi* constituents prevent metabolism of amines from other plants used to make ayahuasca. Following reports of the successful use of *B. caapi* root extracts for treating PD patients in Ecuador, it was shown that, in addition to the MAOI properties, the alkaloids harmine and harmaline

stimulated the release of DA from striatal cells [106]. These compounds would therefore have a double effect in helping improve DA levels, and this may underlie the reputed improvements in PD patients when the extract was taken.

The role of flavonoids in the diet as important antioxidant contributors has received much attention and their neuroprotective properties because of this effect has been demonstrated by several workers. However, they have also been demonstrated to have MAOI activity and this has been proposed as part of the explanation of the use of the common herb St John's Wort, as an antidepressant [107]. This dual role has now been proposed for a variety of flavonoids such as kaempferol 51 from the leaves of *Ginkgo biloba*, a widely used herbal product which has been suggested as a preventative against neurodegeneration [108] (fig. 18). Quercetin 52 has also shown to inhibit

Fig. 18. Flavonoids with monoamine oxidase inhibitory activity.

MAO-B [109] and reverse the effects of induced catalepsy, which mimics the bradykinesia often seen in PD [110]. Tangeretin 53 has been shown to inhibit MAO-B and to cross the blood-brain barrier in a rat model and consequently reduce DA depletion, so it may have therapeutic potential [111]. A compound related to the flavonoids, the polyphenol (-)-epigallocatechin-3-gallate 54, found in green tea, has a similar polyvalent activity which may be sufficient to have a protective effect in PD and other conditions [112]. Since most of these flavonoids occur in reasonably large amounts in common fruits and vegetables, the question is raised as to whether the apparent increase in incidence of neurodegenerative disease is related to some extent with the decline in consumption in the diet of such foods in some sectors of the industrialized world.

Conclusions

Several natural products useful compounds themselves, or have provided lead compounds, for present and potential treatment of Alzheimer's and Parkinson's diseases. Some compounds have reached widespread clinical use, whilst the interest others at present lies more in their providing explanation for traditional uses of plants. Rational drug design according to the characteristics of the receptors involved is now a possibility, and natural mole-

cules can be subjected to 'fine-tuning' by chemical derivatisation and synthesis of analogues to bind more closely and more exclusively to one type of receptor. However, investigation of traditional plants has revealed classes of molecules that have appreciable activity which is unlikely would have been predicted only from receptor studies.

Regarding the treatment of the major diseases involved, it can be said that chemotherapy of AD with cholinesterase inhibitors is unlikely to develop very much further since effective agents such as galantamine have been introduced. Future trends could involve the use of a polyvalent 'cocktail' of drugs which act in different ways by mechanisms such as antioxidant and anti-inflammatory activity and the inhibition of the formation of fibrillary tangles and β -amyloid plaques. Although the introduction of L-DOPA and other dopaminergic compounds over the last three decades has improved the condition of many sufferers of PD, the side effects and their unpredictability of occurrence highlight the fact that more work is needed. In this context also, the exploitation of compounds derived from plants with other mechanisms, such as monoamine oxidase inhibition, may provide a better treatment experience in due course. This may occur in extracts in any case, and clinical trials should be carried out to follow up preliminary results, such as those observed with *Mucuna* bean extract, which indicate an advantage in using an extract over a pure substance.

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