Cholinergic systems in progressive supranuclear palsy

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Summary
Progressive supranuclear palsy (PSP) is a progressive neurodegenerative disease characterized by akinetic-rigid features, falls, a supranuclear gaze palsy and subcortical dementia. Pathologically, there is abnormal accumulation of tau protein. Cholinergic deficits are thought to underlie the postural instability and cognitive impairment of PSP, but trials of cholinergic agonists and cholinesterase inhibitors have failed to show improvement in motor function, quality of life and cognitive impairment. The five cortico-basal ganglia loops, linking functionally related areas of the brain, are damaged in PSP, leading to specific clinical deficits. Cholinergic dysfunction is related to loss of cholinergic interneurons in the striatum, compounded by reduced inputs into the circuits from other cholinergic nuclei, such as the pedunculopontine nucleus and nucleus basalis of Meynert. Normal cholinergic transmission requires the presence of intact cholinergic neurons capable of releasing sufficient acetylcholine, and functional muscarinic and nicotinic receptors. Whilst there is evidence from autopsy and in vivo studies of loss of cholinergic neurons in PSP, the receptor status is unknown. This may be critical to understanding the basis for the poor therapeutic response to cholinomimetics. Symptomatic treatment using cholinergic drugs may thus be improved by more specific targeting of cholinergic receptors or nuclei. There is also evidence that cholinergic agents may have disease-modifying effects. This article reviews the key clinical features of PSP, along with normal basal ganglia anatomy and cholinergic transmission. Cholinergic deficits based on clinical and neurochemical parameters are then discussed, before concluding with suggested future directions for cholinergic treatments.

Keywords: basal ganglia; cholinergic; progressive supranuclear palsy

Abbreviations: ACh = acetylcholine; AChE = acetylcholinesterase; ChAT = choline acetyltransferase; ChEIs = cholinesterase inhibitors; DLB = dementia with Lewy bodies; GABA = γ-aminobutyric acid; LTN = laterodorsal tegmental; nbM = nucleus basalis of Meynert; PPN = pedunculopontine nucleus; PSP = progressive supranuclear palsy


Introduction
Progressive supranuclear palsy (PSP) is a progressive neurodegenerative disease characterized by parkinsonism, falls, a supranuclear gaze palsy and subcortical dementia. It is often misdiagnosed as Parkinson’s disease in the early stages. Unlike Parkinson’s disease, however, the akinetic-rigid features of PSP invariably fail to respond to dopaminergic therapy. Cholinergic deficits are thought to be responsible for the postural instability and cognitive impairment associated with PSP, but to date, cholinergic replacement therapies have been ineffective. The neurochemical basis for this therapeutic refractoriness is uncertain.

In this review we will first summarize the key clinical features of PSP and relevant basal ganglia anatomy, and outline normal cholinergic transmission. Cholinergic abnormalities in PSP will then be reviewed from clinical and neurochemical perspectives. Finally, future research directions will be considered for this system in the context of PSP.

Clinical features of PSP
Classic ‘Richardson’s syndrome’ (Williams et al., 2004) is diagnosed sensitively and specifically by the National
Institute of Neurological Disorders and the Society for Progressive Supranuclear Palsy criteria, which require the presence of falls within the first year of symptoms and a vertical supranuclear eye movement disorder (Litvan et al., 1996a). Mobility difficulties and falls occur due to a combination of bradykinesia, postural instability, retrocollis and supranuclear gaze palsy (Birdi et al., 2002; Nath et al., 2003). Most patients exhibit parkinsonism with symmetrical bradykinesia and rigidity, especially of the axial muscles, although tremor is less common (Birdi et al., 2002; Nath et al., 2003). With time, many patients develop slowing of the vertical saccades, progressing to a restriction of vertical gaze. Bulbar symptoms appear early in the disease course, producing a growing dysarthrophonia and dysphagia. The latter is frequently severe enough to require percutaneous endoscopic gastrostomy feeding.

Cognitive problems and neuropsychiatric symptoms may be early features, manifesting as change in personality, forgetfulness, irritability, anhedonia and depressed mood (Nath et al., 2003). Depression and anxiety occur in approximately 18% of PSP patients (Litvan et al., 1996b). Apathy affects over 90%, although it is often under-recognized and may be mistaken for depression (Litvan et al., 1996b). The prototypic ‘dysexecutive dementia’ present in most PSP patients is evidenced by difficulties with verbal fluency, working memory, concept formation, planning and execution (Grafman et al., 1995; Soliveri et al., 2000). These features are thought to arise from deafferentation of the prefrontal cortex from the basal ganglia. PSP patients also exhibit severe slowness in response time, but neuropsychiatric features associated with other degenerative dementias, such as visual hallucinations and fluctuating cognition, characteristic of dementia with Lewy bodies (DLB), and profound short-term memory loss, typical of Alzheimer’s disease, are rare.

Despite this striking and unmistakable phenotype, there is clinical heterogeneity. Pathologically proven cases, recently described as ‘PSP-parkinsonism’ (Williams et al., 2004), have a more prolonged disease course, and are less likely to present with falls and gaze paresis. Furthermore, up to half may demonstrate a degree of levodopa responsiveness. Accurate clinical diagnosis of PSP-parkinsonism is, unsurprisingly, difficult.

Pathology

Given the clinical heterogeneity of PSP, pathological diagnosis remains the gold standard. Neuronal degeneration and gliosis is found in several subcortical and brainstem nuclei in PSP, particularly the basal ganglia. The most distinctive pathological features are neurofibrillary tangles, neuropil threads and tufted astrocytes, which are found predominantly within the substantia nigra, globus pallidus, subthalamic nucleus, midbrain, pontine reticular formations and, to a lesser extent, the thalamus (Albers and Augood, 2001). The pedunculopontine nucleus (PPN) is also severely affected, showing approximately 60% neuronal loss, with neurofibrillary tangles in the remaining neurons (Zweig et al., 1987; Jellinger, 1988). Although classically a subcortical disease, neurofibrillary tangles and tufted astrocytes have been documented in the frontal cortex, particularly the motor cortex (Wakabayashi and Takahashi, 2004). The pathological inclusions of PSP comprise insoluble aggregates of four-repeat tau phosphoprotein. Tau is important in maintaining neuronal morphology, and mobilizing and stabilizing microtubules involved in axonal transport within the cytoskeleton (Litvan and Hutton, 1998). Tau is also found in the cell body and dendrites. In PSP, tau becomes hyperphosphorylated, but how cell death occurs and whether it is a direct consequence of the tau protein is not known.

Basal ganglia circuits

Five parallel, closed frontal-subcortical circuits link segregated areas of the frontal cortex with the striatum and other basal ganglia, and thalamic nuclei (Alexander et al., 1986). The motor and oculomotor circuits originate in the supplementary motor area and frontal eye fields, respectively. The other three loops are involved in functionally distinct neurobehavioural features. Disruption of projections from the dorsolateral prefrontal cortex, orbitofrontal cortex and anterior cingulate/mediofrontal cortex correlate with executive dysfunction, social behavioural abnormalities and poor motivation, respectively (Cummings, 1993). In PSP, widespread basal ganglia pathology is associated with dysfunction within each of these circuits.

Open circuits connect areas functionally related to the basal ganglia to relevant structures. Cholinergic inputs modulate transmission within these circuits (Fig. 1).

The PPN is a rather diffuse nucleus located in the rostral brainstem tegmentum. It is divided into the pars compacta, containing >90% cholinergic cells, and the pars dissipata,
containing glutamatergic, cholinergic, dopaminergic and adrenergic neurons (Pahapill and Lozano, 2000). The PPN has widespread connections with the basal ganglia, the lower brainstem and spinal cord.

**Striatal organization**

The interaction between dopamine and acetylcholine (ACh) is vital in maintaining striatal function, the key area of neural trafficking within the basal ganglia. Medium spiny projection neurons comprise 90–95% of the striatal neuronal population and are inhibitory via the transmitter γ-aminobutyric acid (GABA) (Graveland and DiFiglia, 1985; Oorschot, 1996). Projection neurons of the direct pathway predominantly express dopaminergic D1 and muscarinic M4 receptors, whilst D2 and M1 receptors are preferentially located on projection neurons of the indirect pathway (Weiner et al., 1990; Bernard et al., 1992; Ince et al., 1997).

The other 5–10% of intrinsic striatal neurons comprise three populations of interneuron, rapidly and burst-firing GABAergic interneurons and larger cholinergic interneurons (1–2%) (Zhou et al., 2003). The cholinergic interneurons, although small in number, have many axon collaterals, enabling them to influence synaptic transmission over a large area. They are tonically active, and stimulation by a neurotransmitter causes the firing of the interneurons to ‘pause’ (Zhou et al., 2002; Pisani et al., 2003). Nigrostriatal, corticostriatal and thalamostriatal afferents all influence interneuronal discharge. The regulation of release of ACh from the interneurons is also, in part, via negative feedback through M2 receptors. ACh from interneurons mediates inhibitory effects at both glutamatergic and GABAergic nerve terminals through an action on presynaptic M2 cholinergic receptors (Calabresi et al., 2000) (Fig. 2).

**Cholinergic neurotransmission**

Cholinergic receptors are divided into muscarinic and nicotinic subtypes. The majority of research in PSP has focused on the muscarinic cholinergic system, although it is likely that nicotinic receptors will also be affected.

**Muscarinic receptors**

Muscarinic receptors are divided into five subgroups, termed M1 to M5. In the brain, M1 receptors are the predominant receptor in the cortex and hippocampus, M2 receptors in the cerebellum and brainstem, while M4 receptors are highly concentrated in the striatum, although there is much overlap (Taylor and Brown, 1999). M3 and M5 receptors are found largely in the peripheral nervous system.

ACh is formed in the presynaptic nerve terminal by the conversion of choline and acetyl coenzyme A to ACh and coenzyme A, catalysed by the enzyme choline acetyltransferase (ChAT). Depolarization of the nerve terminal leads to the entry into the cell of extracellular calcium ions, which in turn leads to ACh release over 200 ms (Taylor and Brown, 1999). In the striatum, ACh binds to postsynaptic M1 or M4 receptors, or presynaptic M2 receptors. Muscarinic receptors are transmembrane spanning and agonist binding initiates a complex cascade of events via a G protein-coupled secondary messenger system. The G protein comprises three subunits: α, β and γ. In the inactive state the α subunit is bound to

Fig. 2 Simplified figure demonstrating presynaptic inhibitory effects of ACh via muscarinic receptor (M) activation at both glutamatergic and GABAergic nerve terminals (based on the figure by Calabresi et al., 2000).
guanine diphosphate (GDP), and the receptor is in a low affinity state (uncoupled from the G protein). Following agonist binding, a conformational change occurs in the receptor that activates coupling to the G protein and the receptor transforms to a high affinity state (coupled). Coupling causes the α subunit to release its GDP to preferentially bind guanine triphosphate (GTP). The GTP-bound subunit interacts with an effector (usually an enzyme) to activate the secondary messenger and the interaction is terminated by GTPase within the α subunit (Fig. 3).

The agonist is released from the receptor, returning it to the low affinity state. Agonist binding to the M1 receptor, and consequent coupling, stimulates phospholipase C, which mobilizes intracellular calcium and has an overall excitatory effect on the nerve cell. M4 binding, and its coupling to the G protein, causes inhibition of adenyl cyclase and calcium channels, producing an overall inhibitory effect on the cell. In the striatum and cortex M2 receptors are located on presynaptic nerve terminals. Like M4, the M2 receptors produce an inhibitory response and once activated prevent neurotransmitter release. Following release of ACh from the receptor, it is rapidly degraded by acetylcholinesterase to prevent desensitization, and then transported into the presynaptic terminal as choline for further transmitter synthesis.

In summary, M2 receptors are markers for presynaptic neurons. ChAT and ACh levels are a measure of intraneuronal ACh formation and release from the terminals, and M1 and M4 receptors are postsynaptic markers.

Nicotinic receptors

Nicotinic acetylcholine receptors are ligand-gated ion channels and are morphologically and pharmacologically distinct from muscarinic receptors. The responses they produce are fast, short-lived and excitatory. They are composed of α and β subunits, the most common type found in the central nervous system being α4β2. Binding of acetylcholine to the receptor causes a rapid influx of cations that leads to depolarization. Nicotinic receptors are widely expressed throughout the brain, with the α4β2 receptors particularly concentrated in the striatum and substantia nigra (Court and Clementi, 1995; Charpantier et al., 1998; Picciotto et al., 1998). Activation of presynaptic nicotinic receptors enhances the release of several neurotransmitters, including dopamine (Zhou et al., 2003).

The cholinergic neurochemistry of PSP

Despite the development of more selective labelling techniques for cholinergic receptors, little research has been directed towards PSP. Post mortem studies using immunocytochemistry and autoradiography provide most of our knowledge (Figure 4).

Striatum

ChAT was reduced by approximately 50% in the caudate, 40% in the putamen, 45% in the nucleus accumbens and 70% in the substantia innominata in nine PSP patients compared with controls (Ruberg et al., 1985). These findings were later supported by an autoradiographic study, which demonstrated a marked reduction in striatal ACh vesicular transporter expression and ChAT activity in 11 PSP patients (Suzuki et al., 2002). Nerve growth factor receptors are localized on nucleus basalis of Meynert (nbM) and intrinsic striatal cholinergic neurons (Villares et al., 1994). Striatal receptors were reduced by 30% in three patients with PSP compared with controls and patients with Parkinson’s disease (Villares et al., 1994). These results suggest loss of striatal cholinergic interneurons. The total number of striatal muscarinic receptors measured by [3H]quinuclidinyl benzilate was similar between controls and PSP patients in one study (Ruberg et al., 1985). However, two further studies using [3H]N-methyl-scopolamine, which also binds to all muscarinic receptors, found an 18–30% reduction in the striatum (Landwehrmeyer and Palacios, 1994; Pascual et al., 1994). Studies using more specific ligands to distinguish between subtypes of muscarinic receptor are needed, but to date, these limited results suggest relative preservation of medium spiny neurons.

Brainstem cholinergic nuclei

Using immunohistochemistry to measure ChAT activity, many small nuclei in the brainstem can be quantitatively measured in post mortem tissue. The PPN and laterodorsal tegmental (LTN) nuclei are the main cholinergic nuclei in the mesopontine tegmentum. Severely decreased ChAT levels
have been demonstrated in the LTN (41%), with a less marked reduction in the PPN (19%) (Kasashima et al., 2003). Low frequency electrical stimulation of the PPN induces positive motor effects in normal non-human primates (Nandi et al., 2002b), and drug-induced PPN stimulation in parkinsonian non-human primates improves akinesia (Nandi et al., 2002a), suggesting that degeneration of these nuclei may contribute to akinesia and gait initiation abnormalities (Pahapill and Lozano, 2000).

Compared with controls, ChAT in PSP is also reduced in the Edinger–Westphal nucleus (69%), the medial longitudinal fasciculus (97%), the superior colliculus (93%) and the interstitial nucleus of Cajal (78%) (Juncos et al., 1991). Involvement of the latter nucleus is thought to result in axial dystonia and gait impairment (Carpenter et al., 1970), whilst the other nuclei mediate eye movements and coordination (Steele, 1972) and pupillary abnormalities (Paffenbach et al., 1972). Cholinergic neurons in cranial nerve nuclei III and IV appear to be spared, consistent with the ‘supranuclear’ gaze paresis. Decreased ChAT has also been found in the lower pontine reticular formation (Malessa et al., 1991).

### Basal forebrain cholinergic nuclei

The nbM, or basal nucleus, lies within the substantia innominata and is one of the main cholinergic nuclei in the brain, with widespread projections to the cerebral cortex and hippocampus. Degeneration within this nucleus has attracted attention in relation to cognitive impairment in Alzheimer’s disease (Mufson et al., 2000), DLB (Londos et al., 2002) and Parkinson’s disease (Bosboom et al., 2004). Marked neuronal loss of 12–55% (Tagliavini et al., 1983, 1984) and reduced ChAT-positive neurons within the nbM are characteristic of PSP (Kasashima and Oda, 2003).

### Thalamus

The mediodorsal nucleus of the thalamus is innervated by many brain regions including the LTN and the substantia innominata, and projects to the prefrontal and premotor cortices (Brandel et al., 1990). ChAT staining in controls reveals areas of densely staining patches surrounded by less dense matrix (Brandel et al., 1990). In five PSP patients, ChAT labelling within the mediodorsal nucleus was reduced by 75% in the matrix and 60% in the patches (Brandel et al., 1991). In rats the medial matrix connects to the orbitofrontal cortex and the densely staining patches to the dorsolateral prefrontal cortex (Cavada et al., 1995).

### Cerebral cortex

ChAT activity was originally reported to be reduced in the frontal cortex in PSP by 21%, together with markedly reduced activity within the substantia innominata (by 70% compared with controls) (Ruberg et al., 1985). A later study reported a modest (20%) but non-significant reduction in ChAT activity in the frontal and temporoparietal cortex in three PSP patients (Whitehouse et al., 1988) These results suggest damage to the innominateocortical cholinergic projections in PSP. Two studies of total muscarinic receptor binding showed similar values between controls and PSP patients in the frontal cortex (Ruberg et al., 1985; Pascual et al., 1994), consistent with the PET study later mentioned.

No study has differentiated between muscarinic receptor subtypes or assessed the function of the secondary messenger system.

### Nicotinic receptors

Despite the involvement of nicotinic systems in Alzheimer’s disease, DLB and Parkinson’s disease, there have been few reports of nicotinic receptor status in PSP. In the only published studies, nicotinic receptors, assessed using [3H]nicotine, were reduced in the frontoparietal, temporoparietal and occipitoparietal cortices in three PSP patients compared with controls, but the numbers were too small for statistical analysis (Whitehouse and Kellar, 1987; Whitehouse et al., 1988).

In contrast, cortical and striatal nicotinic receptors are significantly reduced in Alzheimer’s disease, DLB and Parkinson’s disease (Perry et al., 1990; Rinne et al., 1991; Court et al., 2000; Pimlott et al., 2004; Quik et al., 2004).

Future neurochemical research in PSP needs to be directed towards more specific cholinergic receptor subset measurement, with clinicopathological correlation. The interaction of other neurotransmitters with the cholinergic and dopaminergic systems also needs to be defined.

### Clinical studies of cholinergic abnormalities in PSP

The majority of research in PSP has focused on transmitter abnormalities within the dopaminergic system, with relatively little focus on the cholinergic system. Some cholinergic nuclei, such as those in the brainstem governing eye movements, are so small that neurotransmitter abnormalities within them cannot easily be identified, making quantation difficult. There is some evidence, however, from in vivo and in vitro studies that cholinergic deficits occur in PSP, although little is known about how these abnormalities contribute to the clinical features.

### CSF studies

CSF acetylcholinesterase (AChE) activity is reduced in PSP by one-third compared with control subjects (Atack et al., 1991). The neuropeptide galanin is co-localized with acetylcholine on neurons of the basal forebrain, and, in contrast to the AChE, no significant reduction in this peptide has been found in the CSF of PSP patients compared with controls (Litvan et al., 1992).

### Imaging in PSP

With the development of sophisticated imaging techniques the detection of more subtle and/or specific abnormalities in
PSP has become possible. However, few imaging studies have examined the cholinergic system in PSP (Eidelberg and Dhawan, 2002).

**Structural imaging**
Routine MRI can reveal a distinct pattern of atrophy in PSP, particularly in the midbrain, putamen, globus pallidus and cerebellar peduncles (Schrag et al., 2000). Frontal cortical atrophy has also been demonstrated in several studies (Schrag et al., 2000; Cordato et al., 2002; Brenneis et al., 2004). Using diffusion-weighted imaging to measure water diffusivity across fibre tracts, an increase in apparent diffusion coefficient was found in the lentiform nucleus in PSP, suggestive of neuronal loss and gliosis (Seppi et al., 2003). The sensitivity of these investigations, particularly in early PSP, needs to be established further.

**Magnetic resonance spectroscopy**
Magnetic resonance spectroscopy (MRS) allows in vivo measurement of the concentration of brain metabolites. MRS studies have shown a reduction in the N-acetylaspartate (Naa)/creatine and Naa/choline ratios in PSP patients compared with controls (Davie et al., 1997; Federico et al., 1997a, b; Tedeschi et al., 1997). Inconsistency of MRS in evaluating akinetic-rigid syndromes in general means that this technique is currently of limited diagnostic value (Clarke and Lowry, 2001).

**Functional imaging**
Using PET and N-methyl-4-[11C]piperidyl acetate to measure AChE activity in the cerebral cortex, no statistically significant reduction was demonstrated in PSP patients compared with controls (Shinotoh et al., 1999, 2001). This was in contrast to a significant reduction (−17%) in Parkinson’s disease and Alzheimer’s disease patients (Iyo et al., 1997; Shinotoh et al., 2000). There was, however, a marked reduction in enzyme activity in the thalamus in PSP, which receives cholinergic afferents from the brainstem nuclei including the PPN. PET and 11C-labelled N-methyl-4-piperidyl benzilate has been used to measure total muscarinic ACh receptors (Asahina et al., 1998). PSP patients had no significant changes in binding in any cerebral cortical region. The ligand used does not, however, differentiate between muscarinic receptor subtypes.

**Cholinergic treatment in PSP**
There is pathology in several cholinergic structures in PSP, and cholinergic blockade using scopolamine induces gait disturbance in PSP patients and deterioration in memory in PSP at lower doses than in controls and Parkinson’s disease patients (Litvan et al., 1994; Bedard et al., 1999). It is therefore not surprising that cholinomimetic drugs have been explored for their therapeutic potential in PSP.

In a double-blind, placebo-controlled crossover design, phystostigmine, a central and short-acting cholinesterase inhibitor (0.5–2.0 mg, six times daily for 10 days), did not benefit extra-ocular, parkinsonian or pseudobulbar features.
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in eight PSP patients (Litvan et al., 1989). Some improve-
ments were, however, noted in memory and visuospatial
attention in seven patients (Kertzman et al., 1990). Physos-
tigmine also had no effect upon swallowing or oral motor
function in a 10 day, crossover, placebo-controlled, double-
blind trial (Frattali et al., 1999). The failure of CSF AChE
levels to increase following administration of physostigmine
suggests that the doses used in these studies may have been
insufficient or too short-acting to have any significant central
effect (Attack et al., 1991).

Recently, attention has focused on longer acting choline-
terase inhibitors (ChEIs), in view of their beneficial effects in
Alzheimer’s disease and DLB. Assessment of cognitive func-
tion, motor skills and activities of daily living (ADL) in six
PSP patients at baseline and after 3 months of treatment with
donepezil 10 mg/day revealed no change from baseline in any
of the parameters measured (Fabbriani et al., 2001). The same
dose of donepezil was used in 21 patients in a double-blind,
placebo-controlled, randomized, crossover trial, with each
arm lasting 6 weeks (Litvan et al., 2001). The dose had to
be reduced in three patients due to worsening of motor func-
tion. There was a modest improvement in one of the memory
tests but a significant worsening in motor and ADL scores.
Based on these studies, ChEIs cannot currently be recom-

mended for treatment of PSP.

For ChEIs to produce a therapeutic effect there needs to be
sufficient synthesis and release of ACh from the presynaptic
terminal. Severe cholinergic neuronal loss in PSP may, in
part, account for the lack of response. To overcome this,
direct stimulation of postsynaptic receptors has been
attempted. The non-selective M1 and M2 muscarinic agonist,
RS-86, was given to 10 PSP patients in a 9 week, double-
blind, placebo-controlled, crossover trial. No beneficial
effects were noted upon motor function, eye movements or
cognitive performance, despite electroencephalographic
evidence of enhanced central cholinergic activity upon
sleep architecture (Foster et al., 1989). To our knowledge,
no other cholinergic agonists have been assessed in PSP.

Future directions for cholinergic treatments

Autopsy and some in vivo studies have demonstrated a loss of
cholinergic function within the striatum, thalamus and brain-
stem nuclei in PSP (Striatum-Ruberg et al., 1985; Suzuki et al.,
2002; Villares et al., 1994; Thalamus-Brandel et al., 1990;
Brainstem nuclei-Juncos et al., 1991; Malessa et al., 1991;
Kasashima et al., 2003). The former is presumed to reflect
loss of cholinergic interneurons. The limited information
available on the status of muscarinic receptors has indicated
normal binding within the striatum and cortex. Attempts to
apply effective neurotransmitter replacement therapies have
been unsuccessful. This may be due in part to our lack of
detailed understanding of the neurochemistry of the basal
ganglia and associated areas, but may also be influenced
by the small numbers of patients available to take part in
therapeutic trials.

Cholinergic replacement therapies

The failure of cholinergic replacement therapies in PSP raises
specific questions regarding the underlying neurochemical
abnormalities. First, are the remaining striatal and brainstem
cholinergic neurons unable to produce enough ACh for AChE
inhibitors to act on? Secondly, are the postsynaptic receptors
or a subpopulation of receptors reduced, or is the subsequent
activation of the secondary messenger defective? Thirdly,
despite documented evidence of cholinergic abnormalities
in PSP, is the symptomatology, particularly the cognitive
impairment and gait instability, the result of different neuro-
transmitter abnormalities within the basal ganglia circuitry?
Fourthly, is replacement of neurotransmitters insufficient to
restore the normal physiological function within the basal
ganglia? And finally, rather than a ‘blanket’ approach from
oral cholinergic replacement therapy, do we need to focus on
delivery to specific target nuclei? A better knowledge of the
underlying neurochemical abnormalities in PSP may help to
answer some of these questions.

Extrapolating the results from Alzheimer’s disease trials,
development of selective M1 agonists or M2 antagonists may
improve cognitive function in PSP. M1 agonists were origi-

nally found to improve cognitive function in rats (Fisher et al.,
1991), and M1 receptors are abundant in the hippocampus
and cortex. Clinical trials in Alzheimer’s disease patients
have demonstrated improvement in behaviour and cognition
when treated with xanomeline, a selective M1/M4 agonist,
although its use was limited by dose-dependent adverse
events, particularly gastrointestinal problems (Bodick et al.,
1997a, b; Veroff et al., 1998). When activated, M2 receptors
inhibit ACh release, which may have contributed to the fail-
ure of RS-86, the M1/M2 agonist. Trials of an M2 antagonist
(SCH 72788) in rats increased ACh release in the striatum,
providing a further potential method for cholinergic enhance-
ment (Lachowicz et al., 2001). However, a study of Lu25-
109, a selective M1 partial agonist and M2 antagonist, failed
to improve cognition in a double-blind, placebo-controlled
trial of 496 Alzheimer’s disease patients (Thal et al., 2000).
Overall, these results provide some hope for the use of drugs
acting directly on the muscarinic receptors, although efficacy
may be limited by side-effects.

Direct stimulation of nicotinic receptors could enhance
neurotransmitter release, for example from nigrostriatal dopa-
mimergic neurons and cortical afferents. Unfortunately, pro-
longed stimulation with agonists, rather than the pulsed
physiological stimulation, paradoxically appears to cause
nicotinic receptor antagonism, perhaps through desensitiza-
tion (Zhou et al., 2003). However, allosteric modulation of
nicotinic receptors, such as that which occurs with galant-
amine, may heighten receptor sensitivity by increasing the
probability of channel opening following agonist binding
(Maelicke, 2000; Maelicke et al., 2001). The status of the
nicotinic receptors in PSP is unknown, but one would predict
them to be reduced in the striatum and possibly cortex due to
the selective vulnerability of striatal and cortical afferents.
Nicotine replacement therapy in Parkinson’s disease has produced variable results. Beneficial effects on motor and cognition in Parkinson’s disease patients were reported from small-scale studies of nicotine given by either smoking or transdermal patch (Ishikawa and Miyatake, 1993; Kelton et al., 2000). However, other studies have reported no beneficial effects, with a large proportion of patients withdrawing due to side-effects (Vieregge et al., 2001; Lemay et al., 2003, 2004), or even a worsening of motor symptoms (Ebersbach et al., 1999). In animal models of Parkinson’s disease nicotine appears to protect the dopaminergic neurons against damage from MPTP (Parain et al., 2003). Animal models of Alzheimer’s disease with lesions in the cholinergic basal forebrain show an improvement in cognition when treated with selective nicotine receptors agonists. Furthermore, nicotine appears to reduce production of β-amyloid in Alzheimer’s disease models, suggesting a possible neuroprotective effect (Nordberg et al., 2002; Utsuki et al., 2002). Treatment with nicotine patches improves attentional function in normal non-smoking adults and patients with Alzheimer’s disease (Levin and Rezvani, 2002), and may protect against glutamate-induced cytotoxicity (Akaike et al., 1994). As PSP shares some features of the nigrostriatal degeneration common to Parkinson’s disease and the cholinergic forebrain deficits of Alzheimer’s disease, these studies suggest that the use of nicotine in PSP warrants further investigation. Therapeutic efficacy may, however, be limited by side-effects, lack of receptor specificity or by desensitization of nicotinic receptors.

Cholinergic dysfunction has been demonstrated within the PPN in PSP, but due to its very small size within the densely packed upper brainstem its exact functional role and its interaction with the basal ganglia circuitry is difficult to establish accurately. It has been suggested, mostly based on experiments in rats and non-human primates, that as dysfunc-
tion within the PPN leads to akinesia and gait abnormalities, stimulation of this structure may improve these symptoms (Pahapill and Lozano, 2000). However, deep brain stimulation of the PPN in normal monkeys leads to impairment of postural control at high frequencies and tremor at low frequencies (Nandi et al., 2002b). Excessive GABAergic inhibition from the globus pallidus interna and substantia nigra is thought to contribute to dysfunction of the PPN. In an MPTP-treated monkey model of parkinsonism, infusion of a GABAergic antagonist directly into the PPN alleviated akinesia (Nandi et al., 2002a, c). If these findings can be reproduced in humans it offers hope that either pharmacological or surgical excitation of the predominantly cholinergic PPN may relieve some of the most disabling symptoms of PSP.

**Disease modification therapies**

Potential areas for disease modification include the use of antioxidants and genetic manipulation of tau protein production (Burn and Warren, 2005). Interestingly, some properties of the cholinergic system suggest it may also be a useful target for disease modifying therapies. The potential neuroprotective effects of nicotinic receptor stimulation have already been discussed. Hyperphosphorylated tau protein deposition is common to both PSP and Alzheimer’s disease, and is a major constituent of neurofibrillary tangles, which can disrupt the neuronal cytoskeleton and thereby lead to further degeneration (Goedert, 1993, 2004). Tau phosphorylation is controlled by cell surface cholinergic receptors, as evidenced by M1 receptor agonists producing dephosphorylation of tau in rats (Sadot et al., 1996). Furthermore, in apoE-deficient rats with hyperphosphorylated tau, excess phosphorylation can be reduced using the M1 agonist AF150(S), with an inverse relationship between the levels of phosphorylation and working memory (Genis et al., 1999). This suggests that abnormal tau protein deposition may be a direct result of cholinergic hypofunction, and that dephosphorylation is possible with M1 receptor agonists.

Finally, nerve growth factor (NGF) receptors are located on the basal forebrain and striatal cholinergic neurons and have been shown to be vital in the function of these cells (Perry, 1990). As mentioned previously, these receptors are reduced in the PSP striatum (Villares et al., 1994). NGF, if delivered to specific target areas of the brain, could protect cholinergic neurons from degeneration. NGF does not cross the blood–brain barrier but may be delivered either intravenously, by viral vector or gene therapy, or via the olfactory pathway (Chen et al., 1998). A surgical lesion in the fornix, leading to retrograde degeneration of the cholinergic septal neurons, produces an animal Alzheimer’s disease model. Fibroblasts, genetically modified to secrete NGF and implanted into this rodent Alzheimer’s disease model, can prevent the degeneration of cholinergic neurons (Rosenberg et al., 1988). In non-human primates, recombinant human NGF prevents cholinergic neuronal degeneration (Koliatsos et al., 1991). Similar results are found when using polyethyleneimine as a vehicle for in vivo gene delivery in rats (Wu et al., 2004).

If an effective and safe vehicle for trophic factor delivery in humans can be found, degeneration of the cholinergic neurons in PSP may be halted, although it is unclear whether re-growth of affected neurons is possible.

**Acknowledgements**

N.M.W. is funded by the PSP (Europe) Association.

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