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Cerebral Pharmacodynamics of Physostigmine in Alzheimer's Disease Investigated Using Single-Photon Computerised Tomography

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The effects of physostigmine on patterns of rCBF in patients with pre-senile Alzheimer's disease were studied using ^{99m}Tc-labelled HMPAO SPECT. Regional CBF increased in the left cortex relative to right, with the most significant effect in left frontal and higher frontal regions. Measures of regional brain function, such as SPECT, are an important complement to psychological test batteries in understanding the effects in brain of putative antidementia drugs. SPECT brain imaging could extend our understanding of the action of psychotropic drugs in other major psychiatric illnesses.

There is compelling evidence from many different studies (Sims *et al*, 1983; Rossor *et al*, 1984; Perry, 1986) linking impaired cholinergic neuronal function and memory. Thus, the cholinergic hypothesis of dementia attributes cognitive impairment to defects in the synthesis of acetylcholine (ACh) in central cholinergic neurones (Davies & Maloney, 1976; Davies, 1979). Support for this hypothesis comes from post-mortem neurochemical studies of Alzheimer's disease (AD) which have shown a marked reduction in choline acetyl transferase (ChAT), the rate-limiting enzyme for the synthesis of ACh (Bowen *et al*, 1983), and have also confirmed the reduced production of ACh in such patients (Richter *et al*, 1980). Accordingly there has been considerable interest in pharmacological treatment strategies aimed at facilitating cholinergic function. Although three types of strategy are possible (precursor-loading, inhibition of ACh catabolism, and the use of cholinergic receptor agonists), the more successful results have been obtained using cholinesterase inhibitors to enhance central cholinergic function, and there have been several reports of improved memory/cognition following administration of the anticholinesterase physostigmine (Christie *et al*, 1981; Davis & Mohs, 1982). Another anticholinesterase drug, tetrahydroaminoacridine, which may act in a similar way to physostigmine, also appears to improve memory/cognition in AD (Summers *et al*, 1986; Byrne & Arie, 1989) and has renewed interest in the use of pharmacological strategies to improve cognitive function in Alzheimer patients (Whalley, 1989).

Unfortunately, psychological assessment of improvement in demented patients is difficult because exhaustive batteries take so long to administer while short tests, for example of memory function, may show prominent floor or ceiling effects. Hence there can be many reasons for the failure of such tests to show an improvement, apart from simply lack of drug efficacy. In part, this must contribute to the inconsistent results reported from drug studies (Stern *et al*, 1987). There is, therefore, a need for methods of assessing drug action that are more biologically based and hence less susceptible to the difficulties associated with current psychological test batteries.

Single-photon emission computerised tomography (SPECT) using the blood-flow marker hexamethylpropyleneamine oxime (HMPAO), permits patterns of regional cerebral blood flow (rCBF) to be measured *in vivo*. The reductions in rCBF in dementia are quantitatively related to deficits in neuropsychological function (Hunter *et al*, 1989), and SPECT may therefore provide a useful quantitative measure of drug action in evaluating functional effects on the brain. The aim of this preliminary study was to use SPECT to examine the effects of administration of physostigmine in demented patients.

Method

Study design

The study had two complementary phases. In the first phase, patients' cognitive performance was assessed double blind on three separate occasions, with a different physostigmine dose (0.5 mg, 0.375 mg and placebo) on each. Before and after each test infusion, a short battery

of tests, designed for assessing the psychological effects of drugs, was administered to each subject (Wesnes *et al*, 1987). To avoid practice effects, parallel forms of this test battery were used. Each patient had one practice session and was then tested before and after each infusion. Using this phase of the study, that dose which produced the maximum increase in cognitive performance was determined and used in the second phase. Where no change could be detected the higher dose of the drug was employed.

In the second phase, which was single blind, patients underwent SPECT brain imaging on two separate occasions, once after a saline infusion containing that dose of physostigmine chosen from preliminary testing, and, one week later, with a saline infusion containing no drug. In a test–retest SPECT reliability study, no significant change in ^{99m}Tc-HMPAO uptake was found when patients had a repeat scan after one week (results to be reported). Apart from the presence or absence of active drug, test conditions were identical on each of the two SPECT test occasions. On both test days, patients were pre-treated with methylscopolamine to prevent the peripheral actions of the anticholinesterase. The SPECT-imaging phase of the project is reported and discussed in the present paper.

Seven patients with a diagnosis of probable pre-senile AD were recruited into the study (see Table 1). All patients met agreed criteria for probable AD as laid down by National Institute of Neurological and Communicative Disorders Stroke (NINCDS) and Alzheimer's Disease and Related Disorders Association (ADRDA) (McKhann *et al*, 1984). All patients had their handedness assessed using the Edinburgh Inventory (Oldfield, 1971) and all met criteria for right handedness. Patients and their nearest relative gave informed consent to take part in the study, which was approved by the Ethics of Medical Research Sub-Committee for Psychiatry and Psychology of the Lothian Health Board.

The Cambridge Mental Disorders of the Elderly Examination (CAMDEX) was developed for the diagnosis and measurement of dementia (Roth *et al*, 1986) and was completed for all patients in the study. The CAMDEX contains a section for cognitive assessment (CAMCOG). The 19 items which comprise the Mini Mental State Examination (MMSE; Folstein *et al*, 1975) are incorporated in this section. However, 43 additional items have been added to make the CAMCOG more comprehensive than the MMSE. The total CAMCOG score (maximum 106) was used in the present study as a measure of overall cognitive function.

Table 1
Description of the group of Alzheimer patients studied

Patient no.	Age	Sex	Duration of disease: years	CAMTOTAL score	Physostigmine dose: mg
1	65	F	6.0	39	0.375
2	57	M	1.5	66	0.375
3	55	F	2.5	58	0.375
4	62	F	6.0	40	0.500
5	58	M	6.5	40	0.375
6	60	F	1.5	77	0.375
7	58	F	3.0	55	0.500

All subjects were studied at the Institute of Neurological Sciences, Southern General Hospital, Glasgow, with the tracer ^{99m}Tc-HMPAO. This agent is well suited for SPECT because it shows high fractional uptake into brain but is rapidly metabolised on peripheral recirculation, so that its distribution reflects rCBF (Neirinckx *et al*, 1987). It is retained in the brain for many hours without apparent redistribution and therefore allows a 'snap-shot' image of CBF pattern to be determined at one particular time interval such as during drug infusion.

The HMPAO ('Ceretec', Amersham International plc) was supplied as a freeze-dried kit and reconstituted with sodium pertechnetate solution. Within five minutes of preparation, subjects were administered 2.5 ml (500 MBq) of ^{99m}Tc-HMPAO complex as a single intravenous bolus via a right antecubital vein. The injection of HMPAO was administered 20 minutes after the start of drug infusion, in a quiet room with subjects resting, eyes closed and ears unplugged. Imaging was carried out within 60 minutes using the Novo 810 (Strichman Medical Equipment Inc.), a dedicated brain SPECT system. Subjects were supine on a comfortable tomography table, and a light source was used to align the scanner parallel to the orbitocanthal–meatal (OM) line. The patient's head was supported by polystyrene wedges in a standard configuration. Transverse sectional images at +30, +40, +50, +60 and +70 mm superior to the OM line were constructed. A brain atlas was used to assign the transverse-slice images to a particular anatomical level and to define brain regions with the high resolution of the scanner. There was no particular difficulty in assigning slice levels confidently to particular anatomical regions.

Internal landmarks were used in the final definition of a 'standard' slice, which contained the thalamus and basal ganglia, and of a 'high' slice, which was immediately above the lateral ventricles. Symmetrical paired regions of interest (ROIs) were defined, representing frontal, temporal, posterior temporal, occipital and basal ganglia on the standard slice and high frontal and parietal on the high slice. These are illustrated elsewhere (Hunter *et al*, 1989).

The technique of SPECT imaging with HMPAO provides measurements of the pattern of CBF distribution and not absolute values of CBF. Data normalisation is therefore necessary when making comparisons within groups. Two methods were used in this study: (a) adopting white matter (WM) as a control region and calculating the ratio of mean counts within high frontoparietal cortical regions to mean counts within the WM region; and (b) using an asymmetry index (AI), which is defined as the ratio of counts in each left-sided ROI divided by the counts in the corresponding right-sided ROI.

(a) The amount of HMPAO in frontoparietal grey matter (GM) and WM ROIs were determined from the high slice. A special colour scale was developed to highlight contrast in low-count regions, such as WM, and this was used consistently throughout the study. The same individual (MTH) outlined the GM ROI and a WM ROI medial to high frontal cortex using a 50% contour boundary. This individual was blind as to whether placebo or physostigmine images were being analysed and the order of images analysed was random. The mean count density within

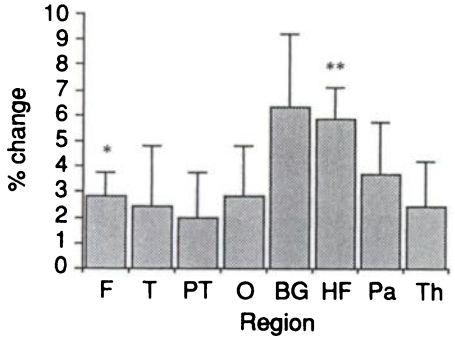

Fig. 1 Histogram showing the percentage change in asymmetry index (left:right ROI ratio) before and after physostigmine, for eight ROIs (F, frontal; T, temporal; PT, posterior temporal; O, occipital; BG, basal ganglia; HF, high frontal; Pa, parietal: Th, thalamus). Comparisons made using Student's paired *t*-test (two-tailed) (*$P<0.05$, **$P<0.005$). Error bars show s.e.m.

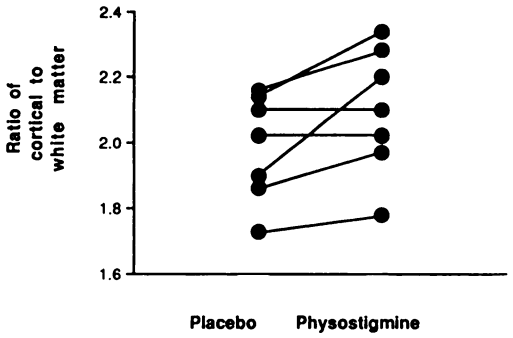

Fig. 2 Graph showing the ratio of counts in frontoparietal cortex to white matter in the higher slice for the seven AD patients after placebo and physostigmine infusions ($P<0.05$).

the WM ROI was measured and then corrected using the equation

$$W' = W - C/3$$

where W' = true count density of HMPAO in WM, W = measured count density of HMPAO in WM (which is affected by scatter), and C = measured count density of HMPAO in adjacent high frontal cortex. This equation was derived empirically using a physiologically realistic phantom which was filled with known concentrations of radionuclide. This procedure was used in order to give more accurate cortical:WM ratios, but the data were also analysed with no correction factor applied and the significance levels for change in this ratio before and after physostigmine remained valid.

(b) The AI is a robust measure of asymmetrical change in pattern. The ROI which is drawn on one side of the brain image is mirrored automatically on the other side and so has exactly the same area. The AI is defined as the ratio of counts in each left-sided ROI to the counts in the corresponding right ROI. In a control group of ten elderly clinically normal volunteers who had resting HMPAO SPECT investigations one week apart, there was no significant change in AI in any of the ROIs outlined.

All patients were given 0.2 mg of methylscopolamine subcutaneously 20 minutes before the start of the physostigmine infusion in order to antagonise only peripheral muscarinic receptors (methylscopolamine does not cross the blood–brain barrier). The infusion of physostigmine was administered intravenously via a forearm cannula and the rate of flow controlled using an IVAC volumetric control pump and delivered over 30 minutes. The dose of physostigmine (Table 1) was delivered in 100 ml normal saline prepared by the Pharmacy Department of the Royal Infirmary of Edinburgh, such that the investigators were blind to the addition or otherwise of physostigmine to the infusion. Electrocardiographic and blood-pressure measurements were monitored throughout the test procedures.

Results

The physostigmine infusions were well tolerated by all patients, and the only significant side-effect recorded was dry mouth, which probably reflects effective peripheral muscarinic blockade by methylscopalamine. Measurements of blood pressure and heart rate remained constant throughout infusions of saline and physostigmine.

After physostigmine administration there were marked changes in asymmetry (Fig. 1). A clear increase in HMPAO uptake on the left side relative to that on the right is seen in all ROIs, with the most significant effect in the frontal and higher frontal regions ($P<0.05$ and 0.005 respectively). This could be interpreted as an increase of CBF on the left side or a decrease in CBF on the right side. However, there was a significant increase ($P<0.05$) in cortical CBF in frontoparietal regions as measured by cortical:white-matter activity ratios (Fig. 2), supporting the hypothesis that cortical perfusion on the left side is increasing. In five patients, cortical:white-matter activity ratios increased after administration of physostigmine compared with saline, while in two patients (1 and 4), there was no change (Fig. 2). The reasons for this are not clear, but it can be seen (Table 1) that patients 1 and 4 have low CAMTOTAL scores and long duration of illness.

In conclusion, these results show that physostigmine increases cortical perfusion, with the most significant increase in left frontal regions.

Discussion

This is the first report of the use of the intravenous radioligand ^{99m}Tc-HMPAO to study the effect of the cholinomimetic drug physostigmine on rCBF in AD. The results of this study are important as they suggest that changes in brain function, produced using specific cholinergic stimulation can be localised *in vivo* using functional SPECT imaging.

It is unlikely that the changes in rCBF pattern observed in this study were simply due to non-specific vasodilatation by physostigmine, for, in a study in

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which CO_2 was used to produce vasodilatation of central vessels, blood flow to cortex *and* white matter increased (Davis *et al*, 1983). As Fig. 2 shows, cortical:white-matter ratios increased after administration of physostigmine in the present study, supporting the view that the increases in flow observed were due to a specific effect on grey matter. Although it is not possible to draw firm conclusions, the lack of response in patients 1 and 4 may be related to severity of neurochemical damage. This may have implications about which Alzheimer patients may benefit from pharmacological intervention.

This method of analysis, using a WM reference area, should only be considered with imaging devices of relatively good spatial resolution (preferably less than 10 mm), such as the NOVO 810. The two principal sources of error were (a) the difficulty in outlining a region which contains only white matter and (b) the effect of scattered radiation emanating from surrounding cortical regions where the radioactivity concentration is much higher, on measured count density within a low-count ROI. These errors were minimised using empirically derived procedures and the correction factor described in the method section, although even before correction for errors in this way, similar significant increases in frontoparietal cortex/white matter were also detected.

It has also been shown in animals that rCBF increases after physostigmine, and that this is paralleled by corresponding increases in regional cerebral oxygen consumption ($rCMRO_2$) (Hoffman *et al*, 1986). This suggests that the coupling relationship between flow and metabolism is maintained after physostigmine. Other animal studies show that after physostigmine there are similar increases in local cerebral glucose utilisation (Nelson *et al*, 1978; Friedland & Meibach, 1981; Dam & London, 1983). The nature of the coupling of rCBF and metabolism is complex and poorly understood, but there is evidence that cholinergic mechanisms may have an important role (Sokoloff, 1959; Scremin *et al*, 1973). The abundant cholinergic innervation of the cerebrovasculature could provide a mechanism whereby changes in cholinergic function are reflected in rCBF changes.

In humans, studies using positron emission tomography (PET) show that rCBF and cerebral metabolism are intimately coupled, under both resting (Frackowiak *et al*, 1981) and activated conditions, and that rCBF closely reflects regional glucose metabolism (Fox *et al*, 1988). Therefore, the changes in rCBF detected after physostigmine in this study probably reflect changes in metabolic function. However, little is known about the effect of cholinomimetics, such as physostigmine, on brain metabolism, as only recently have techniques such as PET and SPECT become available to undertake such studies. Using an older, planar imaging technique for examining cortical flow, Gustafson *et al* (1987) showed that small increases in temporoparietal rCBF occurred after administration of physostigmine. Although limited in being non-tomographic and measuring only surface cortical activity, the results are consistent with the present study.

Post-mortem studies show that there are no consistent or substantial changes in numbers of post-synaptic muscarinic receptors in AD (Davies & Verth, 1978; Palacios, 1982), and this does not appear to be an artefact related to the agonal state or changes after death. SPECT imaging in AD patients using quinuclidinyl benzylate (a ligand for muscarinic receptors) labelled with iodine-123 has suggested that there may be increased numbers of receptors in non-atrophic areas of cortex, but reduced numbers in areas of severe atrophy (Weinberger *et al*, 1989). In relation to the use of drugs that potentiate cholinergic function, such as physostigmine, a key question is how normally do muscarinic receptors *function* in AD?

Unfortunately, *in-vitro* measurement of receptor function in autopsy specimens is technically difficult, and results have been unsatisfactory (Perry, 1986). This emphasises the advantage of our experimental approach of using SPECT to image functional changes *in vivo* after physostigmine.

Alzheimer's disease is usually described as a disorder associated with diffuse atrophy of the cerebral cortex (Tomlinson & Corsellis, 1984). Post-mortem and biopsy studies of Alzheimer patients show that there are marked cholinergic deficits in frontal and parietotemporal cortex (Davies & Maloney, 1976; Bowen *et al*, 1983; Sims *et al*, 1983), but there appears to be no consistent neuropathological or neurochemical involvement of any one hemisphere (Rossor *et al*, 1984). However, only a few post-mortem studies have compared left and right sides in AD, as traditionally one side of the brain is examined neurochemically, and the other morphologically. The few studies that have compared left and right sides, have reported left–right differences in density of plaques and tangles (Moossy *et al*, 1985), and of neurochemical deficits (Rossor *et al*, 1982). Such pathological evidence is consistent with neuropsychological findings that show asymmetrical patterns of cognitive dysfunction in AD, especially in the earlier stages (Haxby *et al*, 1985, 1986; Huff *et al*, 1987; Becker *et al*, 1988).

Similarly, although a symmetrical pattern of regional deficit in blood flow has been observed under resting, non-stimulated conditions with PET (Frackowiak *et al*, 1981) and SPECT (Hunter *et al*, 1989), there is evidence of lateral asymmetry in cerebral glucose utilisation in AD patients with either marked language or visuospatial cognitive deficits (Haxby *et al*, 1985, 1986). It has also been reported that the left hemisphere may be more affected in AD (Rossor *et al*, 1982; Friedland *et al*, 1985; Haxby *et al*, 1985, 1986). This was not confirmed in a recent post-mortem study of AD, although subtle asymmetries in the distribution of plaques and neurofibrillary tangles and cholinergic enzyme activity did occur (Moossy *et al*, 1989). In normal brain, ChAT activity is reportedly higher in left temporal cortex than right, although it was not possible to decide whether this was due to structural or functional differences between the two sides (Amaducci *et al*, 1981). The asymmetric activation seen in the present study probably reflects a functional change, as left:right ratios of CBF changed only after physostigmine, and were not seen during resting conditions.

In pre-senile Alzheimer patients there is marked association of rCBF in left temporoparietal cortex and attention/language function (Hunter *et al*, 1989), and this suggests that the left-sided cortical activation produced by physostigmine in the present study may involve similar left brain cognitive processes. However, in the present study, patients were scanned under resting conditions, and therefore attention and arousal may be the more likely psychological functions involved. Electroencephalographic brain mapping using DUP 996, a phenylindolinone that enhances the release of ACh in brain nerve terminals, shows attenuation of slow activity and increased alpha activity, normally associated with increased vigilance (Saletu *et al*, 1989). Further evidence for the role of the cholinergic system in arousal is that physostigmine antagonises decreases in rCBF and $rCMRO_2$ caused by the sedative benzodiazepine midazolam, probably by a central stimulatory action rather than a competitive effect at the benzodiazepine receptor (Hoffman *et al*, 1986). It is therefore possible that, in the present study, physostigmine may have facilitated increased cortical vigilance by increasing brain ACh levels, and the functional consequence of this was increased blood flow in the left cortex relative to the right.

Although there was a clear trend for an increase in left/right asymmetry in all regions, the magnitude of this effect reached significance only in frontal and high frontal areas (Fig. 1). While the effect of physostigmine could be more specific in frontal areas, an alternative possibility is that after physostigmine, asymmetry increased in many brain regions (Fig. 1), but the measurement of this change only reaches significance in frontal regions because of the better reproducibility of head positioning in that region. This is supported by evidence from reproducibility studies (to be reported elsewhere), in which subjects were scanned on two occasions a week apart under identical conditions, that showed that variance was much less in frontal areas compared with other regions.

One of the difficulties in evaluating putative 'antidementia' compounds, such as physostigmine, is the lack of objective biological measures of effect in the brain. Until recently few valid measures apart from clinical ratings were available. Although clinical indicators are clearly important, there are several disadvantages in their use. For instance, not all patients with AD have the same pattern of functional disabilities; it is this which may partly determine how well a patient is able to perform a given task, and not simply the global severity of Alzheimer pathology. This is perhaps most apparent in rating demented patients with communication or praxic difficulties. Impairment of concentration also affects how well patients perform a test, and consequently the manner in which the test is presented to a patient (e.g. as a whole or in sections), together with the consistency of the rater's testing technique in this regard, will influence the results. Another source of error in the use of rating scales for assessing demented patients is the large component of subjective clinical judgement involved. These difficulties are perhaps even more important when rating scales are used to assess clinical change resulting from therapeutic intervention, as the multiple application of a test to the same patient requires parallel test forms, with increased chances of compounding errors. Finally, patients who are severely affected by the disease and difficult to test may be excluded from clinical studies and thus it is possible that studies biased towards 'testable' patients may be misleading.

It follows that there are important advantages in using biological measures of psychotropic drug action in humans to complement clinical rating measures. Regional CBF, with its intimate relationship to cerebral metabolism, provides an ideal biological measure of pharmacodynamic effect, and one which has been used very successfully in animal models (Sokoloff, 1981). This study demonstrates that such an approach in patients is not only feasible, but provides a useful way of evaluating the central actions of drugs reputed to have beneficial effects in demented patients.

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