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Biochemical measurements in Alzheimer's disease reveal a necessity for improved neuroimaging techniques to study metabolism

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A series of Alzheimer's disease and control brains were dissected to determine the extent of atrophy (based on total protein content) and loss of choline acetyltransferase activity in the cerebral cortex from the entire surface of the diseased brains. The distribution of intensity of pathology so determined is strikingly similar to the degree of hypometabolism as shown by positron emission tomography. It is argued that the hypometabolism can be explained (at least in part) by focal areas of atrophy.

INTRODUCTION

The application in the 1960s of methods of biochemical analysis to human samples led to important advances in the understanding of brain diseases, particularly the inborn errors of lipid metabolism (Sweeley & Klionsky, 1963; Suzuki & Suzuki, 1970) and Parkinson's disease (Hornykiewicz, 1963). Later it was found that some brain constituents are sensitive to epiphenomena, notably to the way in which patients die. Thus it was recognized that some techniques carried out *in vitro* and developed for assaying normal brain are of limited use for investigating diseased tissues (Spillane *et al.*, 1977). The assay of neurosurgical samples (biopsies) carried out to circumvent such problems (Sims *et al.*, 1981), clearly aids in the interpretation of deficits of neurotransmitters in Alzheimer's disease detected *post-mortem* (Palmer *et al.*, 1988) but has produced somewhat unexpected observations for metabolism (summarized later). These and new data reported here on the distribution of cortical atrophy based on the assay of biochemical markers, are discussed in relation to metabolic measurements by positron emission tomography. These, it is argued, are affected by atrophy.

EXPERIMENTAL

Tissue samples

The investigation was performed on brains that had previously been dissected (Najlerahim & Bowen, 1988). Briefly, frozen hemispheres were processed from six female controls (four right, two left hemispheres) and six female subjects with Alzheimer's disease (three right, three left hemispheres). The mean age in years of 78.5 (range 61–87) for controls and 81.5 (range 73–89) for Alzheimer subjects and also the post-mortem delay in hours of 38 ± 21 for controls and 43 ± 27 for Alzheimer subjects, were not significantly different (Student's t test). Subjects with Alzheimer's disease had a clinical diagnosis of that disorder together with senile plaque and neurofibrillary degeneration in the cortex consistent with a diagnosis of Alzheimer's disease; clinical and histopathological data indicated that the control group was free of gross psychiatric or neurological disturbance. No subject had evidence of more than slight cerebrovascular disease. There was no selection of subjects according to disease severity.

A Bauknecht commercial meat slicer was used to cut 5 cm thick coronal sections from the frozen brains which had been transferred from -70 to -20 °C 12 h before cutting. Areas of the cortex were identified visually according to brain anatomy and dissected on a mechanically cooled cold tray (-20 °C). The accumulated tissue from each area was finely chopped and mixed to create an homogeneous sample of grey matter, free from meninges and white matter, and weighed, all as previously described (Najlerahim & Bowen, 1988).

Determination of protein content and choline acetyltransferase activity

Cell-free homogenates of representative aliquots of the weighed portions of grey matter from the various anatomically defined regions were assayed for protein content and choline acetyltransferase activity by the methods of Lowry *et al.* (1951) and Fonnum (1975), respectively. Protein content and enzyme activity were expressed per entire region (g of protein/g wet wt. per g wet wt. of region and enzyme activity/g of protein per g of protein of region) for each brain hemisphere.

Statistical methods

Results are expressed as means $\pm$ S.D. Group means were compared using the two-tailed Student's t test except for three comparisons where there was a significant difference in variance ($P < 0.05$, Fisher F test) so the Mann–Whitney U-test was used here.

RESULTS AND DISCUSSION

Cerebral atrophy is a major abnormality in Alzheimer's disease. In post-mortem studies this has been estimated by weighing the brain, comparing brain volume with cranial capacity and by measuring either cortical thickness or length (Hauw *et al.*, 1986). Most neuropatho-

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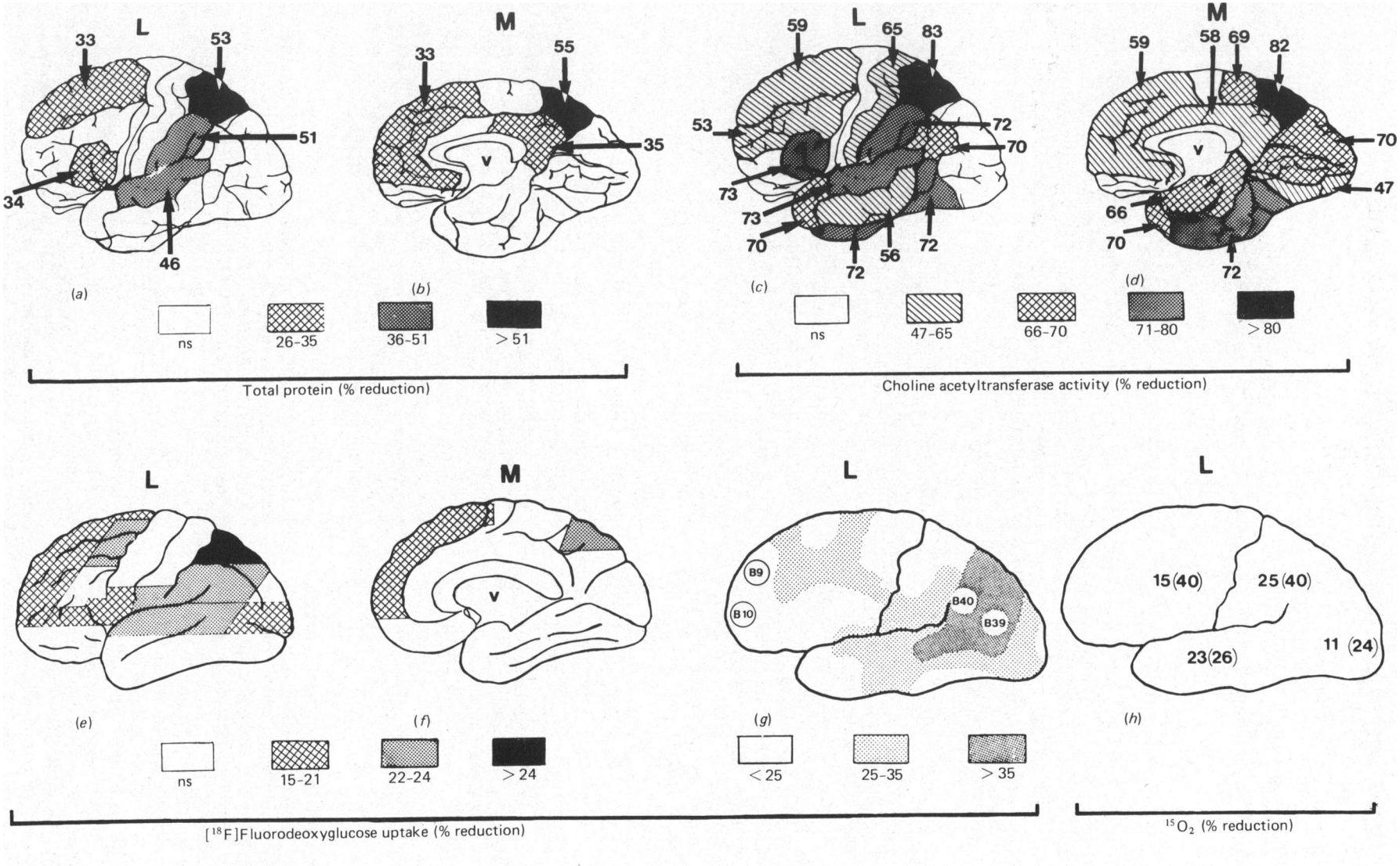

Fig. 1. Maps of biochemical measurements made *in vitro* and *in vivo* in the cerebral cortex in Alzheimer's disease

A shaded area identifies a significant reduction, the arrows identify the percentage reduction in some of the biochemical measurements; ns is non-significant. L and M identify the lateral and medial brain surfaces, V is the third ventricle, (*a*)–(*d*) are mean protein contents or enzyme activities in Alzheimer's disease as percentage reduction compared with controls (from data in Table 1). (*e*) and (*f*) (Index of glucose uptake for control; corresponding Alzheimer's disease value/control value) × 100 (calculated from published uptake values expressed per unit mass as is the convention for such measurements; Horwitz *et al.*, 1987). (*g*) is reproduced with the permission of authors (Tamminga *et al.*, 1987) and publisher. In (*h*), the numbers are taken from published data (Frackowiak *et al.*, 1981) for mildly demented subjects; the numbers in parentheses are per cent reduction for severely demented patients.

Table 1. Total protein content and choline acetyltransferase activity of cortical grey matter from various areas of the brain

Grey matter was dissected and weighed from various entire anatomically defined regions of the cerebral cortex [data reported by Najlerahim & Bowen (1987)] and representative aliquots were assayed. The values below are means ± S.D. of individual values for six controls and six subjects with Alzheimer's disease. BA identifies samples in the region of various Brodmann areas. Significance of difference from control (two-tailed Student's t test): $^{a}P < 0.05$; $^{b}P < 0.02$; $^{c}P < 0.01$; $^{d}P < 0.001$.

Brain region	Total protein content (g/region)		Choline acetyltransferase activity (nmol/min per region)	
	Control	Alzheimer's disease	Control	Alzheimer's disease
Frontal lobe				
Superior frontal gyrus	1.68±0.40	1.12±0.34[a]	123.0±39.2	50.7±28.8[c]
Middle frontal gyrus	0.87±0.13	0.87±0.24	72.9±24.9	34.3±18.7[a]
Inferior frontal gyrus	0.52±0.29	0.60±0.25	58.3±29.2	30.2±22.8
Orbito frontal gyrus	1.21±0.61	1.01±0.46	101.8±64.5	54.9±22.8
BA44,45	0.47±0.09	0.31±0.11[a]	53.8±23.6	14.5±11.4[c]
Pre-central gyrus	0.78±0.28	0.71±0.21	54.3±34.3	36.1±23.7
Para-central gyrus	0.18±0.06	0.18±0.07	17.2±6.7	10.3±5.3
Parietal lobe				
Post-central gyrus	0.63±0.23	0.49±0.21	29.9±14.4	10.5±6.0[b]
Para-central gyrus	0.24±0.09	0.23±0.08	34.8±17.4	10.8±6.0[a]
Superior parietal lobule	1.08±0.27	0.51±0.17[c]	70.2±12.5	12.1±7.8[d]
Supramarginal gyrus	1.07±0.32	0.53±0.23[b]	95.9±30.7	27.3±15.3[d]
Angular gyrus	0.40±0.16	0.26±0.14	29.8±10.7	9.1±9.8[b]
Precuneus	0.66±0.18	0.30±0.17[b]	40.2±10.4	7.3±4.8[d]
Temporal lobe				
Superior temporal gyrus	0.52±0.12	0.28±0.05[c]	44.0±11.2	12.1±5.4[d]
Middle temporal gyrus	0.61±0.17	0.44±0.09	39.0±11.6	17.1±7.4[c]
BA20	1.02±0.37	0.65±0.21	68.4±14.6	19.2±10.6[d]
Insula	0.19±0.04	0.16±0.03	45.0±8.2	14.5±9.3[d]
Posterior temporal (BA37)	0.46±0.16	0.35±0.13	39.9±14.3	11.3±7.9[c]
Anterior temporal (BA38)	0.66±0.15	0.46±0.18	68.3±19.5	20.6±6.9[d]
Parahippocampal gyrus	0.45±0.13	0.29±0.14	46.5±17.0	16.0±21.9[a]
Occipital lobe				
Superior occipital lobule	0.59±0.18	0.56±0.22	34.2±10.3	20.0±13.2
Lateral occipital lobule	0.48±0.15	0.43±0.18	34.2±10.5	19.6±12.5
Cuneus	0.81±0.12	0.70±0.20	44.3±14.1	13.5±13.6[c]
Lingular gyrus	0.39±0.11	0.35±0.19	26.1±3.2	13.9±10.7[a]
Cingulate gyrus				
Anterior	0.14±0.05	0.12±0.03	15.4±4.9	6.5±6.0[a]
Posterior	0.39±0.08	0.25±0.06[b]	31.1±6.1	12.8±6.8[c]

logical assessments highlight the temporal lobe as being most affected. Atrophy of this lobe has already been objectively assessed by determining the wet weight and protein content of entire lobes and the 20% reduction (Bowen *et al.*, 1979) agrees with brain volume measurements (Hubbard & Anderson, 1981). This biochemical approach has now been extended to various anatomical regions of the cortex (protein content, Table 1; see also Figs. 1*a* and 1*b*). The cortical areas have also been analysed for choline acetyltransferase activity (Table 1; see also Figs. 1*c* and 1*d*), loss of which is a well recognized feature of the condition (Palmer *et al.*, 1988). The superior parietal lobule was slightly more affected than the most affected areas of the temporal lobe. These findings are not unique as uptake of $^{15}O_2$ and [^{18}F]-fluorodeoxyglucose show foci of reduced uptake in the region of the parietal lobe (Figs. 1*e*–1*h*). The topographical distribution of the loss of protein and reduction in uptake of [^{18}F]fluorodeoxyglucose closely coincide. Both are markedly affected in the precuneus (superior medial parietal cortex) and superior temporal gyrus as well as the superior parietal lobule. Other regions are spared except for frontal gyri. (Uptake into the occipital lobe is not consistently affected.) The distribution of loss of cortical choline acetyltransferase activity was similar to that of the other measures but the loss of enzyme activity was much more marked and occurred in many more areas. Loss of this neurotransmitter marker probably results from a loss of cholinergic terminals of cell perikarya in the subcortex. Losses of nerve cells in the neocortex have also been reported but there is, in general, a near absence of change in the density of neurones here (Hauw *et al.*, 1986), yet the cerebral cortex is often the most atrophic. This shrinkage has been attributed to "collapse of the neuropile" (Hauw *et al.*, 1986) brought about by loss of major neuronal structures (e.g. pyramidal neurones) that are organized in a columnar manner (Procter *et al.*, 1988*a*).

These reductions determined by positron emission tomography in markers of oxygen and glucose uptake have generally been interpreted as indicating diminished functional activity in disease-affected brain tissue. The

maximal respiratory rates (measured in the presence of an uncoupling agent) of cortical tissue of Alzheimer patients *in vitro* are not reduced and under some conditions, which may be more relevant to the normal physiological state, this biopsy tissue also exhibits elevated respiratory rates (Sims *et al.*, 1987) as well as increased $^{14}CO_2$ production from [U-^{14}C]glucose (Sims *et al.*, 1981). The measurements made *in vivo* are thought to be affected by metabolically inert cerebrospinal fluid (Chawluk *et al.*, 1987) as well as the functional activity of the brain. The spatial resolution of positron emission tomography scanners also probably does not allow a distinction between the metabolism of cerebral cortex and closely adjacent white matter which has a lower rate of metabolism. Chawluk *et al.* (1987) have argued that complex volumetric analyses on a regional basis are needed to decide whether apparent local metabolic alterations in dementia (Figs. 1*e*–1*g*) are complicated by atrophy. The most straightforward interpretation of the present observations (Figs. 1*a*–1*d*) together with the measurements made *in vitro* is that the reported hypometabolism in Alzheimer's disease (Figs. 1*e*–1*h*) can be explained (at least in part) by focal atrophy. Other explanations that need to be considered include a selective vulnerability to death (McGeer *et al.*, 1986) of major neuronal structures that are hypermetabolic *in vitro* but have reduced neuronal activity (and metabolic demand) *in vivo* because of disproportionately more inhibitory mechanism (e.g. γ-aminobutyric acid synapses; Cudennec *et al.*, 1987; Procter *et al.*, 1988*b*).

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