Short report

Tetrahydrobiopterin metabolism in the temporal lobe of patients dying with senile dementia of Alzheimer type

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SUMMARY There is a defect in tetrahydrobiopterin metabolism in brains from subjects with senile dementia of Alzheimer type compared to age-matched controls. This defect results in lowered total biopterin concentrations in brain. Brains from subjects with senile dementia of Alzheimer type retain their ability to synthesis neopterin and have normal dihydropteridine reductase activity, indicating a specific loss of ability to convert dihydroneopterin triphosphate to tetrahydrobiopterin.

The synthesis of dopamine and noradrenaline in the brain is dependent upon the activity of tyrosine hydroxylase, and this in turn is regulated by the concentration of the hydroxylase cofactor, 5, 6, 7, 8-tetrahydrobiopterin (BH_4).¹ Recent reports have suggested a defect in BH_4 metabolism in senile dementia of Alzheimer type; biopterin concentrations in serum² and CSF³ are lowered in patients with senile dementia of Alzheimer type compared to age-matched controls, suggesting a role for BH_4 in the pathology of senile dementia of Alzheimer type. Williams *et al*⁴ have shown that biopterin concentrations in CSF fall with age, suggesting a decrease in the tetrahydrobiopterin available for neurotransmitter biosynthesis. We have studied BH_4 synthesis, dihydropteridine reductase activity and biopterin and neopterin concentrations in the temporal lobe of patients dying with senile dementia of Alzheimer type and in age-matched controls, and report the results here.

Method

Brain samples

Samples of temporal lobe (Brodmann area 20) removed at necropsy from eight normal subjects and eight subjects

with senile dementia of Alzheimer type were obtained from the MRC brain bank. These brain samples were matched for age, drug therapy and time to necropsy. Brain samples were stored at -70°C until required. Twenty per cent homogenates of brain samples in 0.1M tris buffer pH 7.6 were prepared and centrifuged at 100 000 g for 40 minutes. Supernatants were used for all measurements. Protein was measured by the biuret method. Each brain sample was assayed for dihydropteridine reductase, BH_4 synthesis activity, total neopterin and total biopterin.

Assay of dihydropteridine reductase

Dihydropteridine reductase was assayed by the method of Craine *et al*.⁵ Each incubation contained 10^{-4}M nicotinamide adenine dinucleotide, reduced form (NADH) 10^{-3}M H_2O_2 , 8 μg horseradish peroxidase, $2.5 \times 10^{-4}\text{M}$ sodium azide, 10^{-4}M 6, 7-dimethyl-tetrahydropterin, in 0.05 M tris buffer pH 7.6, and 0.02 ml of brain extract in a total volume of 1 ml.

Measurement of BH_4 synthesis

BH_4 synthesis was measured by the method of Fukushima *et al*.⁶ Each incubation contained $6 \times 10^{-3}\text{M}$ guanosine triphosphate (GTP), $3 \times 10^{-3}\text{M}$ adenine dinucleotide phosphate, reduced form (NADPH), 10^{-3}M MgCl_2 and 0.1 ml of brain extract in $7.5 \times 10^{-4}\text{M}$ tris buffer pH 8.0, in a total volume of 1 ml. Under these conditions the synthesis was linear for a period of 3 h and the concentrations of NADPH and GTP were not rate limiting. For controls incubations were carried out in the absence of GTP and/or NADPH. Incubation was for 3 h at 37°C in the dark. The reaction was stopped with 2 ml of 0.1 M HCl and reduced biopterins oxidised with I_2 .⁷ Total biopterin

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was measured by (HPLC) high performance liquid chromatography.

Measurement of total neopterin and total biopterin concentrations in brain homogenates was by HPLC after acid-iodine oxidation.⁷

Results and discussion

Recovery of BH₄ synthesising activity and dihydropteridine reductase from control brain and senile dementia of Alzheimer type brains was 90–95% showing that, as in the rat,⁶ BH₄ synthesising enzymes and dihydropteridine reductase are in the cell supernatant fraction.

Kinetic measurements on the synthesis of BH₄ in control brains showed that the concentrations of GTP and NADPH used for the incubation were not rate-limiting. Because senile dementia of Alzheimer type brains showed little or no synthesis of BH₄, it was not possible to carry out kinetic measurements on these samples. Determination of Km for dihydropteridine reductase in control brain extracts and senile dementia of Alzheimer type brain extracts gave Kms of 1.2×10^{-3} M and 1.5×10^{-4} M for 6, 7, dimethyltetrahydropterin and NADH respectively. These values are similar to those for the sheep liver enzyme⁵. There was no correlation between time to necropsy and either BH₄ synthesis activity or dihydropteridine reductase in either group.

BH₄ metabolism was impaired in the brains of subjects with senile dementia of Alzheimer type compared to age-matched controls (table). Synthesis of BH₄ was significantly lower in senile dementia of Alzheimer type brains (0.046 ng biopterin/mg protein/h) than in the control brains (0.616 ng biopterin/mg protein/h) ($p = 1\%$ Wilcoxon sum of ranks). In the control group there was evidence for a decrease in the ability to synthesise BH₄ with age, the youngest brain (age 44 years) having a synthesis activity of 1.05 ng biopterin/mg protein/h, six brains aged 70–80 years having a mean of 0.75 ng biopterin/mg protein/h and one brain (age 89 years) having synthesis activity of 0.11 ng biopterin/mg protein/h. Dihydropteridine reductase activity in temporal lobes from subjects with senile dementia of Alzheimer type was higher than that in temporal lobes from control subjects, but the elevation was not significant. Measurement of total neopterin and total biopterin in temporal lobes gave a neopterin/biopterin ratio of 0.55 in control subjects and 5.23 in subjects with senile dementia of Alzheimer type. In temporal lobes from subjects with senile dementia of Alzheimer type the total biopterin concentration had fallen, but the total neopterin concentration had risen compared to age-matched controls.

The results presented here demonstrate a deficit

Table BH₄ synthesis, dihydropteridine reductase activity, total neopterin concentration and total biopterin concentration in temporal lobes from subjects with senile dementia of Alzheimer type and age-matched controls. Number of subjects in brackets. Results are expressed as mean $\pm$ standard deviation.

	Normal controls Mean age 71.8 $\pm$ 12.7 yr	Subjects with SDAT Mean age 77.0 $\pm$ 6.8 yr
Synthesis (8) ng biopterin/mg protein/hr	0.616 $\pm$ 0.511	0.046 $\pm$ 0.102
DHPR (8) nmol NADH/mg protein/min	229.4 $\pm$ 11.0	295.5 $\pm$ 143.0
Total biopterin (7) ng/mg protein	0.902 $\pm$ 1.03	0.232 $\pm$ 0.22
Total neopterin (7) ng/mg protein	0.496 $\pm$ 0.35	1.237 $\pm$ 0.94
neopterin/biopterin ratio	0.55	5.33

in BH₄ in temporal lobes of subjects with senile dementia of Alzheimer type. This deficit is not due to lack of dihydropteridine reductase, but a failure to synthesise BH₄ and is in agreement with the results of Morar *et al.*,³ who have shown that total biopterin concentrations were significantly lowered in the CSF of subjects with senile dementia of Alzheimer type compared to age matched controls. BH₄ synthesis activity in senile dementia of Alzheimer type brains was 7.5% of that found in control brains. This decrease was greater than found for other enzymes in senile dementia of Alzheimer type.⁸ The high level of synthesis activity in the one younger subject in the control group compared to older controls is indicative of a decrease in the ability to synthesise BH₄ with increasing age, a result which is in agreement with the work of Williams *et al.*,⁴ who have shown that BH₄ concentrations in CSF decrease with increasing age. The alteration in the neopterin to biopterin ratio in senile dementia of Alzheimer type compared to controls shows that the failure to synthesise BH₄ in senile dementia of Alzheimer type is not due to a defect in GTP cyclohydrolase, the enzyme that converts GTP to dihydroneopterin triphosphate, but that the defect occurs in the biosynthetic pathway after dihydroneopterin and demonstrates a defect in BH₄ synthesis *in vivo*. In this respect the defect is comparable to that found in those neonates who are unable to make BH₄ due to a genetic defect.⁹ These children are able to synthesise dihydroneopterin but lack the ability to convert dihydroneopterin to BH₄. Since senile dementia of Alzheimer type brains retain the ability to synthesise dihydroneopterin, but lack the ability to make BH₄, and dihydropteridine reductase is unimpaired, it seems unlikely that these results are due to neuronal loss in senile dementia of Alzheimer type brains.

In conclusion, the temporal lobes from subjects with senile dementia of Alzheimer type had impaired ability to synthesise BH_4 compared to age-matched controls. The defect in BH_4 synthesis was not due to loss of brain cells, but to a loss of ability to convert dihydroneopterin to BH_4 . The results suggest that the capacity to synthesise BH_4 decrease with increasing age, and that loss of the capacity to synthesis BH_4 may contribute to the development of Alzheimer's disease.

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