

## Letters

### The effect of age on tetrahydrobiopterin metabolism in the human brain

Sir: The availability of the hydroxylase cofactor, tetrahydrobiopterin, is a regulatory factor in catecholamine biosynthesis.<sup>1</sup> BH<sub>4</sub> levels are maintained in the cell by de novo synthesis from guanosine triphosphate (GTP) and a salvage pathway requiring dihydropteridine reductase (DHPR).<sup>2</sup> The biosynthetic pathway requires three enzymes: GTP cyclohydrolase, phosphate eliminating enzyme and sepiapterin reductase in that order.<sup>3,4</sup>

It has been reported that biopterin levels in CSF decrease with age<sup>5</sup> suggesting that BH<sub>4</sub> metabolism is altered in the CNS in normal ageing. We now report diminished BH<sub>4</sub> synthesis and DHPR activity in the ageing brain.

Human brain samples from the frontal cortex (Brodmann area 9) and temporal cortex (Brodmann areas 20/21) obtained at necropsy were supplied by courtesy of Dr Reynolds and his colleagues at the Brain Bank, Cambridge, and Dr Altmann at the London Hospital, Whitechapel, London. High speed supernatants of tissue homogenates were prepared in 0.107 M Tris buffer pH 7.6 and used in all assays. BH<sub>4</sub> synthesis<sup>6</sup> and DHPR activity<sup>2</sup> were determined for the frontal cortex and temporal cortex. GTP cyclohydrolase activity<sup>7</sup> and sepiapterin reductase activity<sup>8</sup> were determined in the temporal cortex. All activities were measured on a protein base-line using the Biuret method for protein determination. Pearson correlation coefficients were calculated for age and enzyme activity.

BH<sub>4</sub> synthesis significantly declines with age in the frontal cortex ( $r = 0.590$ ,  $p < 0.05$ ) and temporal cortex ( $r = 0.523$ ,

$p < 0.05$ ) (table). DHPR activity significantly declines with age in the frontal cortex ( $r = 0.722$ ,  $p < 0.01$ ) and temporal cortex ( $r = 0.575$ ,  $p < 0.01$ ). Sepiapterin reductase activity and GTP cyclohydrolase activity show no correlation with age in the regions examined.

BH<sub>4</sub> levels in the CNS decline in normal ageing.<sup>5</sup> We find that BH<sub>4</sub> synthesis and DHPR activity are reduced in the ageing brain suggesting that decreased cofactor availability may limit noradrenergic activity in the ageing brain. It may be argued that reduced BH<sub>4</sub> metabolism is the result of the neuron loss which occurs in the locus coeruleus with age.<sup>9</sup> However, GTP cyclohydrolase activity and sepiapterin reductase activity show no correlation with age. Therefore we propose that the salvage pathway and biosynthetic pathway are diminished in the ageing brain and further suggest that the lesion in BH<sub>4</sub> synthesis is at the level of phosphate eliminating enzyme.

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Accepted 5 June 1986

Table Correlation between enzyme activity and age in the frontal cortex and temporal cortex

	Age Range Years	n	r	p
BH <sub>4</sub> synthesis				
frontal cortex	40-87	14	-0.590	0.05
temporal cortex	40-87	16	-0.523	0.05
Dihydropteridine reductase				
frontal cortex	40-87	14	-0.722	0.01
temporal cortex	40-89	19	-0.575	0.01
GTP cyclohydrolase				
temporal cortex	40-85	9	-	ns
Sepiapterin reductase				
temporal cortex	40-85	8	-	ns

### T cell subsets in AIDS: relationships between peripheral blood and cerebrospinal fluid

Sir: A profound deficiency in immune function underlies the ongoing epidemic of the acquired immune deficiency syndrome (AIDS). A major feature of the immune deficiency in AIDS is a lowering of the ratio of helper to suppressor T lymphocytes in the peripheral blood.<sup>1</sup> Neurological complications are observed in over 30% of the cases and the CNS may be frequently and early involved in AIDS.<sup>2</sup> CSF studies have revealed inflammatory features, such as pleiocytosis, raised IgG index and oligoclonal bands but quantitative changes in T helper or T suppressor lymphocytes have not yet been reported.

We have analysed T lymphocyte subsets in CSF and blood of three AIDS patients with central nervous system manifestations and one patient with AIDS-related complex. T-cell-surface phenotypes were performed with Leu-3a (Beckton Dickinson) to detect

helper/inducer T cells and with Leu-2a (Beckton Dickinson) to detect suppressor-cytotoxic T cells. Peripheral blood mononuclear cells were separated through a Ficoll-Paque gradient and characterised by an indirect immunofluorescence assay as previously described.<sup>3</sup> Cytological preparations were made from fresh CSF samples with a Shandon Cytospin. The cells were stained with haematoxylin and immunocytochemistry with monoclonal antibodies was performed using the Vectastin ABC kit (Vector Laboratories). At least 100 cells were counted per slide.

The table shows the values for T cell subsets and the helper/suppressor ratio for each patient. The lowering of the ratio of T helper to suppressor T lymphocytes in PBL from a mean of 2.20 in control subjects to 0.46 in patients with AIDS is also observed in the CSF, with a decrease from a mean of 2.18 in other neurological disorders (2 polyradiculoneuritis, 1 meningoradiculoneuritis, 5 low back pain, 1 cerebrovascular disease and 1 dementia) to 0.33 in patients with AIDS or AIDS-related complex. These differences are statistically highly significant ( $p < 0.001$ ) in both the CSF and the peripheral blood. The marked decrease in the percentage of T helper cells found in AIDS is often accompanied by an absolute or a relative increase in suppressor cells and this was observed in most of the patients. One patient had a Burkitt lymphoma with meningeal involvement at the time of examination and over 80% of the cells in the CSF reacted with the B cell antigen Leu 12.

The finding of severely reduced percentage of T helper cells in the CSF of AIDS patients and in the AIDS-related complex is of interest aside from its possible diagnostic implications. It has been shown that the AIDS

virus (HIV) was detected in the brain of AIDS patients and it has been suggested that HIV could be implicated as a possible cause of AIDS encephalopathy.<sup>4</sup> Furthermore, the presence within the blood-brain barrier of a specific immunoglobulin to HIV in patients with neurological complications of the AIDS or the AIDS-related complex has been documented.<sup>5</sup> Though the decrease in T helper lymphocyte in the CSF may reflect an altered ratio in the peripheral blood, it may also suggest actual destruction of the helper subset in the brain. Migration and sequestration of activated T cells from peripheral blood into the central nervous system could lead to a persistent virus infection. This could play an important role in relation to long term immuno-pathological consequences. It is of interest to note that many viruses involved in CNS infections also infect lymphocytes and that lymphotropic viruses have recently been implicated in the pathogenesis of multiple sclerosis,<sup>6</sup> a chronic neurological disease associated with disturbances in the relative proportions of T cell subpopulations.

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Accepted 19 June 1986

**Hiccups and vomiting as initial manifestations of multiple sclerosis**

Sir: Hiccuping and vomiting are such rare features of multiple sclerosis that they are not mentioned in standard texts on the disease,<sup>1,2</sup> although they have been described in isolated case reports.<sup>3,4</sup> We report a case in which both hiccups and vomiting were the presenting features of the illness, though this was not recognised at the time.

A 28 year old woman from the Dominican Republic was seen at various emergency rooms and clinics in Rhode Island for a period of 6 months prior to admission, for complaints of intermittent sustained hiccuping and mild vomiting for periods lasting up to 16 days. Physical examination, complete blood count and electrolytes were all normal. She returned to the Dominican Republic, where a neurological evaluation was normal. Two months prior to admission she developed headache and diplopia in association with worsening and now continuous hiccups, all of which resolved following a course of ACTH given for an unsubstantiated diagnosis of adrenal insufficiency.

She was admitted to the Roger Williams General Hospital with recurrent diplopia and a severe headache located behind the eyes, but without hiccups or vomiting. Other symptoms included diminished sensation on the left side of the face and difficulty chewing on the left side. Past medical history was remarkable for hypothyroidism treated with thyroid replacement, and an episode of "diet pill" toxicity. Cranial nerve examination was

Table

Clinical stage	CSF cells/mm <sup>3</sup>	suppressor cells			helper/suppressor ratio*
		helper cells	% of total cells		
AIDS, Burkitt lymphoma with meningeal involvement	441†	CSF	8	4	0.50
		PBL	55	38	0.69
AIDS, cryptococcal meningitis	74	CSF	55	13	0.24
		PBL	53	20	0.38
AIDS, encephalopathy	3	CSF	64	0	0.00
		PBL	70	10	0.14
AIDS-related complex	8	CSF	7	4	0.57
		PBL	31	20	0.65

\*Mean ± SD for normal in peripheral blood is 2.20 ± 0.17 (n = 44) and mean ± SD for other neurological disorders in CSF is 2.18 ± 0.25 (n = 10).  
†Over 80% of the cells in the CSF reacted with the B-cell antigen Leu 12.