

Monoamine metabolite concentrations in lumbar cerebrospinal fluid of patients with histologically verified Alzheimer's dementia

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SUMMARY Concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) were determined in lumbar cerebrospinal fluid (CSF) from control subjects and patients of both presenile and senile age with histologically verified Alzheimer's dementia. CSF HVA increased with age in control but not in Alzheimer patients. HVA and 5-HIAA in the CSF of presenile Alzheimer patients was lower than that of age matched control subjects.

Alzheimer's dementia, now regarded as a disease of senium as well as pre-senium, presents as an impairment of higher mental function¹ and is characterised histologically by large numbers of neuritic (senile) plaques and neurofibrillary tangles in the neocortex and hippocampus.² A further characteristic is a substantial decline of presynaptic cholinergic function in the neocortex.³ Although the involvement of other neurotransmitter systems is not well established, evidence implicating both a cortical⁴⁻⁸ and a subcortical⁹⁻¹¹ dysfunction of monoamine neurotransmitter systems has emerged.

Indirect assessment of cerebral monoamine function in memory disorders by analysis of metabolites in cerebrospinal fluid (CSF) has identified a specific alteration in Korsakoff's psychosis.¹² Similar studies in Alzheimer's dementia have yielded conflicting results, with some authors reporting reduced concentrations of 5-hydroxyindoleacetic acid (5-HIAA)¹³ and homovanillic acid (HVA)¹³⁻¹⁸ while others¹⁹⁻²¹ find no change. It seems likely that these discrepancies are partly due to differences in diag-

nostic criteria since the diagnosis of Alzheimer's dementia was not verified by neurohistological examination in any of these studies.

The present investigation compared the concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-HIAA and HVA in the CSF of a group of histologically verified presenile Alzheimer patients with that of a group of age-matched controls, apparently free of neurological disease. Both groups have also been compared with a small group of histologically confirmed Alzheimer patients of senile age. A preliminary report of part of this study has previously been published.²²

Materials and methods

CSF was analysed from 34 patients with Alzheimer's dementia, 25 (10 male, 15 female) were of presenile age (less than 70 years) and nine (four male, five female) were of senile age (at least 70 years). All patients had a clinical diagnosis of Alzheimer's dementia. Brain tissue was examined at diagnostic craniotomy in 12 and at necropsy in 22 patients. Plaque and tangle formation was of an intensity consistent with a diagnosis of Alzheimer's dementia in all cases. All the biopsy-verified patients were drug free at the time of lumbar puncture, whereas 14 of the necropsy verified patients were undergoing treatment with phenothiazines and/or butyrophenones. The control group consisted of 38 patients undergoing investigation for a variety of peripheral and spinal cord complaints. Only patients over 50 years of age (10 male, 15 female) were used to compare with Alzheimer patients. Patients with evidence

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of neurological disease or disc lesions were excluded.

MHPG, 5-HIAA and HVA standards (Sigma Chemical Co, Poole, UK) were stored as 100 µg/ml aliquots at -70°C and diluted appropriately before use. CSF was removed from the lumbar subarachnoid space following overnight fast and frozen at -70°C until analysis. Samples were filtered using 15 µM microfilters (Bioanalytical Systems Inc, West Lafayette, USA) and separated by reverse phase chromatography using a 4.6 × 150 mm C₁₈, 5µM column protected by a 4.6 × 60 mm pre-column (Ultrasphere, Altex Scientific Inc, USA). The mobile phase comprised: 94% 200 mM Na acetate/acetic acid, 50 µM ethylenediaminetetraacetic acid, pH 4.96 (all Analar grade chemicals from British Drug Houses); 6% HPLC grade methanol (Rathburn Chemicals Ltd, Walkerburn, UK). Prior to use, the mobile phase was passed, under vacuum, through a 0.5 µM filter (FHUP 04700, Millipore Ltd, London). A reciprocating pump (11 VA, Altex) maintained a flow rate of 1.3 ml/min. An electrochemical detector (LC4A, Bioanalytical Systems Inc) together with a glassy carbon electrode (TL5, Bioanalytical Systems Inc) were used at a potential of 750 mV. Preliminary studies identified MHPG, 5-HIAA and HVA peaks in CSF by (a) spiking with a small volume of an appropriate concentration of standard and (b) comparing the electrode response profile with varying potential in CSF with that of standards. Peaks were subsequently identified by comparing retention times in CSF with that of a standard solution. CSF monoamine metabolite concentrations were determined by comparing the peak heights of standards with duplicate 100 µl injections of CSF. Electrode response increased linearly from 0 to 100 ng/ml and the sensitivity (defined as amount detectable when signal/noise = 2) was calculated to be 80 pg for MHPG and 5-HIAA and 140 pg for HVA. Interfering peaks obscured MHPG in one and 5-HIAA in three cases. Statistical differences between groups was assessed by a two-tailed Student's *t* test. Correlations were examined using the Pearson product moment.

Results

CSF concentrations of all three metabolites were independent of patient gender. Alzheimer patients undergoing neuroleptic drug treatment had 5-HIAA values 39% higher than drug free Alzheimer patients of similar age. MHPG and HVA were not significantly affected by drug therapy (table 1). The content of HVA in the CSF correlated with age in control ($r = 0.39$, $n = 38$, $p < 0.02$; fig)

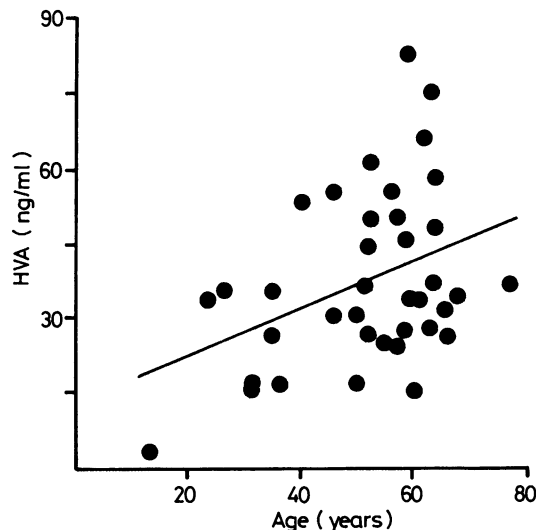


Fig Correlation between homovanillic acid concentration in the CSF of control patients and age ($n = 38$, $r = 0.39$, $p < 0.02$).

but not Alzheimer ($r = 0.09$, $n = 34$) patients. Age did not correlate with either MHPG or 5-HIAA.

Table 2 shows that in comparison with age-matched control subjects, the HVA content in the CSF of presenile Alzheimer patients was reduced by 28% and that the 5-HIAA content of drug-free Alzheimer patients was reduced by 25%. MHPG was unchanged. 5-HIAA and MHPG were unaltered in senile Alzheimer patients. The elevation of HVA content with age in control subjects invalidates direct comparison between mean values for senile Alzheimer patients and the younger control subjects. Extrapolation from the figure, however, suggests that 50 ng/ml is an appropriate control value. This indicates that homovanillic acid in the CSF of Alzheimer patients of senile age is reduced by 42%.

Table 1 Effect of neurotropic drug treatment on monoamine metabolite concentrations in the CSF of patients with Alzheimer's dementia. Results are mean \pm SD, with number of patients in parenthesis

	Age (yr)	Concentration in CSF (ng/ml)		
		HVA	5-HIAA	MHPG
Drug treated	64 \pm 4 (13)	35.5 \pm 24.6 (13)	21.2 \pm 6.6 (11)	7.2 \pm 1.4 (13)
Drug free	69 \pm 12 (21)	28.1 \pm 13.4 (21)	15.3 \pm 5.7 (20)*	8.3 \pm 2.4 (21)

* $p < 0.05$, Student's *t* test.

Table 2 Concentration of MHPG, 5-HIAA and HVA in the CSF of histologically-verified presenile and senile Alzheimer patients compared to a group of control subjects. There is no selection of data except for 5-HIAA where only results for patients free from neuroleptic treatment are included. Results are mean \pm 1 SD with number of patients in parenthesis.

	Age (yr)	Concentration in CSF (ng/ml)		
		HVA	5-HIAA	MHPG
Controls	60 \pm 6 (25)	42.3 \pm 17.1 (25)	21.4 \pm 8.4 (23)	7.2 \pm 2.3 (25)
Alzheimer's dementia (presenile)	63 \pm 5 (25)	30.5 \pm 16.1 (25) [†]	16.0 \pm 6.0 (16) [*]	8.3 \pm 2.2 (25)
Alzheimer's dementia (senile)	79 \pm 11 (9) [‡]	29.1 \pm 17.8 (9) [§]	13.7 \pm 5.7 (4)	6.4 \pm 1.0 (4)

*Results significantly different from control: * $p < 0.05$, [†] $p < 0.02$, [‡] $p < 0.001$.

§Identifies data for which the control value was inappropriate (see text).

Discussion

A pronounced cranio-caudal HVA concentration gradient,²³ together with a large reduction in the HVA content in lumbar fluid of patients with spinal canal blockage,^{24–27} suggests that HVA in the lumbar subarachnoid space is mainly of cerebral origin. Moreover, the highest CSF concentration of this metabolite is found in the lateral ventricles,²⁸ so it is likely that HVA originates from the caudate nucleus. Analysis of this region at necropsy has suggested an age-dependent decline in dopamine function²⁹ which contrasts with the age-dependent increase in the content of HVA in CSF found in this and other studies.^{18, 30, 31} This change in CSF HVA is, therefore, likely to be a consequence of either increased egress from brain or reduced outflow from CSF.

The finding of a reduced concentration of HVA in the CSF of histologically verified Alzheimer patients may reflect the increase in ventricular volume that accompanies cerebral atrophy. This is unlikely, since patients with normotensive hydrocephalus have greater than normal concentrations of HVA in the lumbar fluid.^{32, 33} An alternative explanation is that there is an alteration in the activity of dopamine neurons in the caudate nucleus of Alzheimer patients. Necropsy analysis supports this view, with a reported 38% decline in the concentration of HVA in this region in Alzheimer's dementia,⁹ but without a change in dopamine content. Investigation of patients with Parkinson's disease,³⁵ suggest that such an alteration is unlikely to have a clinical effect.

5-HIAA also displays a cranio-caudal concentration gradient²³ although analysis of patients with interrupted CSF flow reveals no more than a slight reduction in 5-HIAA content of lumbar fluid.^{24–27} Thus it is unclear whether the present finding of reduced 5-HIAA content in the CSF of Alzheimer's dementia patients is related to a cerebral or spinal cord dysfunction. Spinal cord pathology is not a feature of Alzheimer's dementia so it seems reasonable

to attribute the reduction in the content of 5-HIAA in CSF to cerebral changes.

Diminished noradrenergic neuronal function in the neocortex^{4–7} together with extensive cell loss in the locus coeruleus^{6, 10, 11} suggest an involvement of ascending noradrenergic pathways in the pathogenesis of Alzheimer's dementia. The absence of a change in the content of MHPG in the CSF of Alzheimer patients presented here indicates a sparing of descending coeruleo-spinal neurons.^{27, 36, 37} in this disorder. The pathogenesis of Alzheimer's dementia is, therefore, likely to differ from Korsakoff's psychosis where reduced CSF concentrations of MHPG, together with a correlation between this metabolite and amnesic symptoms, have been reported.¹² Such selectivity in Alzheimer's dementia suggests that neocortical noradrenergic dysfunction is not primarily due to an intrinsic defect in the locus coeruleus.

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