 Reduced plasma oestrogen stimulated neurophysin and delayed response to oestrogen challenge in Alzheimer’s disease

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SYNOPSIS Plasma concentrations of oestrogen stimulated neurophysin (ESN) were reduced in 28 patients with Alzheimer’s disease (AD) compared with 14 age-matched controls, 16 patients with other presenile dementias and 12 patients with major depressive disorder. The ESN response to oestrogen challenge was delayed in 10 AD patients compared with 7 age-matched controls. Reduced basal and oestrogen stimulated plasma ESN may be related to impaired responsiveness of the hypothalamo-neurohypophysial neurons and/or a reduction in the amount of pituitary ESN available for release. Plasma ESN measurements may be of value for excluding the diagnosis of AD in patients with dementia who present before the age of 65.

INTRODUCTION

In Alzheimer’s disease of presenile onset (AD), plasma concentrations of oestrogen stimulated neurophysin (ESN) were decreased compared with age-matched controls, other presenile dementias and major depressive disorder (Christie et al. 1987). ESN is derived from the oxytocin prohormone which is processed during its transport from cell bodies in the paraventricular and supraoptic nuclei of the hypothalamus to nerve terminals in the neurohypophysis where ESN and oxytocin are coreleased into the circulation (Reichlin, 1985). ESN is preferred to oxytocin as a marker of the function of the magnocellular oxytocin hypothalamo-neurohypophysial neurons because ESN has a relatively long half-life in plasma (Whalley et al. 1987).

The present study had two aims. First, to measure plasma ESN in a larger number of patients than previously studied (Christie et al. 1987) to determine whether plasma ESN concentrations could be used as a diagnostic test for AD. Second, to determine whether the ESN response to oestrogen challenge was abnormal in AD and, if so, whether this could also be used as a biological marker to assist in diagnosis.

METHOD

There were four age-matched groups: patients with AD, other presenile dementias, major depressive disorder and healthy controls. Thirty-one patients with AD of presenile onset were diagnosed and followed up for one to five years (McKhann et al. 1984; Blackburn & Tyrer, 1985; Christie et al. 1987). The other dementias group comprised 11 patients with multi-infarct dementia, three patients with Huntington’s disease, one patient with Pick’s disease and one patient with a dementia probably due to demyelination. Patients in whom multi-infarct dementia was diagnosed showed multiple infarcts on CT scanning and/or an ischaemic score of eight or more (Hachinski et al. 1975). Twelve patients met Research Diagnostic Criteria (RDC) for major depressive disorder (Spitzer et al. 1978) and criteria for endogenous or probably endogenous subtypes; six of these patients were psychotic. Twenty-one controls with no significant medical history and no evidence of cognitive impairment were used. The age and sex distribution of the groups are shown.
Table 1. Description of patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>M</th>
<th>F</th>
<th>Mean ± s.D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal plasma ESN</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>28</td>
<td>11</td>
<td>17</td>
<td>62 ± 5</td>
<td>53   70</td>
</tr>
<tr>
<td>Other dementias</td>
<td>16</td>
<td>9</td>
<td>7</td>
<td>61 ± 8</td>
<td>44   70</td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>12</td>
<td>4</td>
<td>8</td>
<td>58 ± 6</td>
<td>48   65</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>3</td>
<td>11</td>
<td>56 ± 6</td>
<td>48   65</td>
</tr>
<tr>
<td>Oestrogen stimulation test</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>62 ± 5</td>
<td>59   70</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>65 ± 7</td>
<td>59   77</td>
</tr>
</tbody>
</table>

Ten of the 28 AD patients who had basal plasma ESN concentrations studied, took part in the oestrogen stimulation test at a later date. The control subjects were different in the basal and stimulation studies. Of the 28 AD patients studied, data for 16 were published in Christie et al. 1987.

in Table 1. Patients were investigated while inpatients in the research ward, and were not permitted to smoke during the study. Similarly control subjects were also requested not to smoke for the duration of the study. All patients and control subjects were free of neuroleptic and antidepressant medication for at least three months and all consented to the study, which was approved by the Ethics of Medical Research Subcommittee for Psychiatry and Psychology, Lothian Health Board.

For the estimation of basal plasma ESN concentrations an indwelling cannula was inserted. Blood samples were taken at 07.00, 07.30 and 08.00 h (morning), 15.00, 15.30 and 16.00 h (afternoon) and 23.00, 23.30 and 24.00 h (evening), to take account of the diurnal and pulsatile pattern of hormone secretion (Christie et al. 1987). For the oestrogen stimulation test, blood was taken at 09.00 h just before oral administration of 100 μg of mestranol (Syntex Pharmaceuticals Ltd., Maidenhead, UK). Further blood samples were taken 24, 48 and 72 h after ingestion of mestranol (Amico et al. 1981). Blood samples were collected in lithium heparin-coated tubes containing 100 KIU Trasylol (Bayer UK Ltd.) and the separated plasma stored at −40 °C.

Plasma ESN concentrations were determined using a radioimmunoassay kit from The National Hormone and Pituitary Program (NHPP), Baltimore, Maryland, USA, as used previously in this laboratory (Christie et al. 1987; Whalley et al. 1987). Briefly, both the reference preparation and the antisera to ESN were donated to NHPP by Dr A. Robinson, University of Pittsburgh, Pennsylvania, USA, and have been described elsewhere (Robinson et al. 1975). There is little cross-reactivity between antisera for ESN and nicotine stimulated neurophysin, (NSN), and no increase in plasma ESN concentration occurs when subjects smoke tobacco (Robinson et al. 1975), suggesting that the restrictions on smoking in this study may have been unnecessary. Anti-rabbit gamma-globulin (Scottish Antibody Production Unit, Carluke, Scotland) at a final dilution of 1/100 was used as a second antibody. The sensitivity of the assay (90% B/B o) was 80 pmol/l plasma. The mean intra- and inter-assay coefficients of variation of two quality control samples were less than 10%.

The data were not normally distributed. Comparisons were therefore made using Kruskal–Wallis one-way analysis of variance and differences between groups were identified using Mann–Whitney U tests. Correlations were tested using Spearman’s rank correlation coefficient. P values < 0.05 were taken as significant.

RESULTS

Plasma ESN concentrations in the three blood samples taken in the morning, in the afternoon and in the evening, did not differ significantly. Comparisons were therefore made using mean morning, mean afternoon and mean evening values. There was no difference in ESN concentrations between male and female subjects in the total population or in the AD group, and no relationship, in this age range, between ESN
FIG. 1. Plasma concentrations of ESN (mean ± S.E.M.) in Alzheimer’s disease (AD) ■, other dementias ◼, major depressive disorders △ and control subjects □. The values in AD were significantly different compared with those in other dementias (morning $P < 0.05$, afternoon $P < 0.02$ and evening, $P < 0.05$), major depressive disorders (morning $P < 0.01$, afternoon $P < 0.02$, evening $P < 0.02$) and controls (morning $P < 0.05$, afternoon $P < 0.05$ and evening $P < 0.01$).

Concentrations and age. Plasma ESN concentrations were significantly lower in the morning, afternoon and evening in AD compared with other dementias, major depressive disorder and control subjects (Fig. 1). Morning, afternoon and evening ESN concentrations did not differ significantly within each group, taking males and females together and separately. All AD patients had morning ESN concentrations less than 170 pmol/l whereas 33% of patients with major depressive disorder, 50% of patients with other dementias and 36% of control subjects had plasma ESN concentrations above the upper limit of the AD range.

The ESN response to mestranol ingestion was delayed in the AD patients (Fig. 2). The AD patients had lower plasma ESN concentrations ($P < 0.01$) at 24 h compared with control subjects, and the increment (24 h minus baseline values) in plasma ESN was also significantly less in the AD patients ($P < 0.05$). The maximal ESN response to oestrogen was related to basal ESN concentrations in AD patients ($r_s = 0.80, P < 0.01$) and control subjects ($r_s = 0.83, P < 0.02$).

CONCLUSION

These data confirm our previous findings that plasma ESN concentrations are significantly reduced in AD (Christie et al. 1987) and show
that reduced basal ESN concentrations are associated with a delayed ESN response to oestrogen challenge. Since no AD patient had plasma ESN values higher than 170 pmol/l, and since 50% of patients with presenile dementias other than AD had concentrations of ESN higher than 170 pmol/l, ESN measurements in plasma may be of value for excluding the diagnosis of AD in patients with dementia who present before the age of 65.

Decreased plasma concentrations of ESN in AD patients could reflect decreased release of ESN from nerve terminals in the pituitary which could be due to reduced storage in these terminals or disordered ESN synthesis and transport in the hypothalamo-neurohypophysial tract. In senile dementia of the Alzheimer type (ATD), the concentrations of ESN and oxytocin in the hypothalamus (Yates et al. 1989) and mean cell area and nuclear and nucleolar diameters of oxytocin immunoreactive cells in the paraventricular and supraoptic nuclei (Fliers et al. 1985; Hoogendijk et al. 1985) were the same as in age-matched controls. These reports argue against a deficiency in the synthesis of ESN and oxytocin in senile dementia of the Alzheimer type and possibly in presenile AD. However, the pituitary content of ESN is about 50% lower in Alzheimer-type dementia (ATD) compared with that in matched control subjects (Yates et al. 1990), and the lower plasma concentrations of ESN and the reduced ESN response to oestrogen in AD may reflect a reduction in the amount of ESN available for release. The reduced plasma levels of ESN could be related to reduced plasma levels of dehydroepiandrosterone sulphate, a precursor of oestrogen, in AD patients (Sunderland et al. 1989). Decreased levels of estrogen have been observed in plasma from post-menopausal women with probable senile dementia of the Alzheimer type (Fillitt et al. 1986). Since in the monkey very few neurons in the paraventricular and supraoptic nuclei concentrate oestrogen, that is, contain oestrogen receptors (Pfaff et al. 1976), oestrogen control of ESN and oxytocin release in humans is probably mediated by neurons which modulate the activity of oxytocin neurons and not by a direct action on the oxytocin neurons themselves. The delayed ESN response to oestrogen challenge in AD could be due to decreased responsiveness of these modulatory neurons. Since acetylcholine stimulates oxytocin release (Reichlin, 1985), the delayed ESN response to oestrogen may reflect the cholinergic deficit present in the brains of AD patients (see Hardy et al. 1985). The reduced response of plasma vasopressin to osmotic stimulation and metoclopramide (Norbiato et al. 1988) and physostigmine (Raskind et al. 1989) in AD patients suggests that decreased responsiveness of both vasopressin and oxytocin-containing hypothalamo-neurohypophysial neurons may be a feature of AD.

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REFERENCES


