GHRH-induced GH response in patients with senile dementia of the Alzheimer type

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Abstract. To clarify the functional state of the somatotropinergic system at the hypothalamo-hypophyseal level in senile dementia of the Alzheimer type, the GHRH test was performed in three groups of subjects: a) healthy elderly subjects; b) early onset senile dementia patients; and c) late onset senile dementia patients. Intravenous administration of GHRH(1–44)NH₂ (100 μg) elicited a marked plasma GH response with a maximum peak (709.54 ± 259.0 pmol/l; P < 0.005) 60 min after injection in patients with early onset senile dementia, but no significant response was detected in the other two groups. Electroencephalographic recording showed that GHRH modifies brain bioelectrical activity, decreasing frequency (0.52 ± 0.15 Hz) and increasing amplitude (8.25 ± 4.5 μV) of the electroencephalogram basic rhythm. The evaluation of mental performance and behaviour with a battery of different tests for mental assessment revealed that GHRH induces transient clinical changes in psychomotor behaviour. According to these results, it seems likely that the somatostatin deficiency reported in senile dementia of the Alzheimer type may account for the enhanced GHRH-induced GH response observed in patients with early onset senile dementia. In consequence, the GHRH test might constitute a useful antemortem marker for senile dementia of the Alzheimer type if the present results can be replicated in early stages of the disease.

The neurochemical characteristics of early and late onset senile dementia of the Alzheimer type (SDAT) can be clearly demonstrated in post-mortem studies in which it is possible to observe that cholinergic and somatostatinergic deficits are more pronounced in patients with an early onset of the disease (Rossor et al. 1984). In advanced stages, clinical assessments show that the course of the disease is more rapid and prominent in younger patients. Since dementia may be attributed to various etiologies of which SDAT accounts for at least 50% of the cases, the search for antemortem markers capable of establishing an early differential diagnosis for identifying potentially reversible or treatable causes of dementia is becoming an extremely important matter (Hollander et al. 1986).

SDAT was originally considered a disorder of cortical cholinergic innervation (Coyle et al. 1983), but at present it is believed that this pathological process is a multisystem disorder (Price et al. 1986). The most relevant peptidergic abnormalities in SDAT involve a clear deficit in cortical somatostatin (Davies et al. 1980; Rossor et al. 1984; Beal et al. 1986) and CRH (Bissette et al. 1985; De Souza et al. 1986). Other peptidergic...
and monoaminergic systems are also affected (see Beal et al. 1986; Cacabelos et al. 1986a, 1987b). As a consequence of the overall deterioration of somatostatin neurons, it has been postulated that in SDAT there is an additional dysregulation of GHRH which is partially compensated by multiple hormone and neurotransmitter interactions (Cacabelos et al. 1986a). However, there is no information about the GHRH-induced GH response in SDAT. This is in part due to the fact that most authors agree that the GH response to GHRH is attenuated in elderly subjects (Guillemin et al. 1984; Shibasaki et al. 1984) and old laboratory animals (Sonntag et al. 1983; Ceda et al. 1986; Cacabelos et al. 1986b). The assessment of this neuroendocrine response may help to elucidate the functional state of the somatotropinergic system at the hypothalamo-hypophyseal level in patients with brain somatostatin deficits. Furthermore, new insights into a differential diagnostic approach should be possible if reliable conclusions are achieved.

The rationale for giving GHRH to these patients is indicated by the fact that this neuropeptide has proved to be effective in enhancing food intake (Vaccarino et al. 1985), locomotion, and, to a lesser extent, learning in a dose-dependent manner in rats (Cacabelos et al. 1987a,c). Since these behavioural parameters are impaired in SDAT, it would be worthwhile to investigate whether GHRH may be of utility as a palliative treatment (Cacabelos et al. 1986a, 1987a). The second matter to take into consideration is the necessity of establishing accurate antemortem markers for SDAT (Holland et al. 1986). In this regard, the determination of basal levels of cortisol (Davis et al. 1986) and GH (Christie et al. 1986) appears to be a useful diagnostic tool. Another approach to the problem should be to use neuroendocrine tests (Cacabelos et al. 1987b) in order to formulate a differential diagnosis of SDAT. As mentioned above, somatostatin is the most affected neuropeptide in the brain of patients with SDAT; the functional evaluation of the somatotropinergic system, by means of the GHRH test, would theoretically be the most appropriate way to acquire more diagnostic criteria for the disease. Hence, the main purpose of this paper is, primarily, to study the effect of GHRH on GH secretion so as to clarify whether the deficiency of somatostatin present in SDAT can influence the GHRH-induced GH response which is apparently absent in normal elderly subjects; and secondarily, to investigate to what extent a single dose of GHRH is able to induce behavioural changes in SDAT patients.

Subjects and Methods

The subjects were divided into three groups: a) controls (N = 9): 5 females and 4 males (age = 70.10 ± 2.76 years; range = 66–75); b) inpatients with early onset senile dementia of the Alzheimer type (EOSDAT) (N = 10): 5 females and 5 males (age = 65.00 ± 3.31 years; range = 57–69); and c) inpatients with late onset senile dementia of the Alzheimer type (LOSDAT) (N = 10): 5 females and 5 males (age = 75.70 ± 3.77 years; range = 70–82). All the SDAT patients met the DSM-III criteria for primary degenerative dementia and rigorous diagnostic criteria for SDAT. As EOSDAT patients were considered those whose insidious memory loss or deterioration of cognitive functions began before the age of 60 and had a progressive course of more than 2 years. In LOSDAT, the onset of the disease occurred after the age of 60. The degree of severity of the dementia was assessed with Hasegawa's Dementia Rating Scale (DRS) (Hasegawa & Inoue 1974), Folstein's Mini-Mental State (MMS) Test (Folstein et al. 1975), and a modified model of the Brief Cognitive Rating Scale (BCRS) and Functional Assessment Stages (FAST) of Reisberg (Reisberg et al. 1985). Concomitant diseases other than SDAT were ruled out under a careful screening of the subjects with CT scan, EEG, ECG and complementary laboratory data. Current diagnosis or history of DSM-III major affective disorder or schizophrenia as well as history of endocrine disease were criteria for exclusion. All of the control subjects were free of psychiatric and endocrine illness. Informed consent was obtained from relatives of the SDAT patients and from the volunteers serving as controls. All subjects included had been drug-free from a minimum of 7 days before testing.

A 21-gauge indwelling venous needle with an attached three-way stopcock was inserted into the antecubital vein. Baseline blood samples for GH determination were collected immediately before testing. GHRH(1-44)NH2 (SM-8144; Sumitomo Pharmaceutical Co Ltd, Osaka, Japan) was then injected as an iv bolus (100 µg), and blood samples were obtained at 15, 30, 45, 60, 90, and 120 min after injection for determination of GH concentrations in plasma. Blood samples were treated as previously described (Cacabelos et al. 1985) and the plasma GH levels were measured by RIA using a commercial GH-RIA-Kit (Dainabot Co Ltd, Tokyo). The intra- and inter-assay coefficients of variation were 6.9% and 10.6%, respectively.
GHRH-induced GH response in elderly subjects (●) and patients with early (■) and late onset senile dementia of the Alzheimer type (▲). *P < 0.005 vs basal level (0) (mean ± SD). **P < 0.005 vs control (●).

Results

GHRH induced a marked increase in the levels of plasma GH in from 30 to 90 min after injection with a maximum peak (709.54 ± 259.50 pmol/l, *P < 0.005) at 60 min in EOSDAT (Fig. 1). This response was absent in both LOSDAT and control subjects, in whom plasma GH increased slightly, but never reached a significant level over basal

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**Table 1.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control (females)</th>
<th>Control (males)</th>
<th>EOSDAT (females)</th>
<th>EOSDAT (males)</th>
<th>LOSDAT (females)</th>
<th>LOSDAT (males)</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>46.81 ± 42.72</td>
<td>48.63 ± 41.80</td>
<td>87.27 ± 69.09</td>
<td>90.45 ± 75.90</td>
<td>49.54 ± 45.91</td>
<td>43.18 ± 44.55</td>
</tr>
<tr>
<td>15</td>
<td>140.45 ± 71.81</td>
<td>100.45 ± 85.01</td>
<td>101.36 ± 45.45</td>
<td>122.72 ± 87.27</td>
<td>92.72 ± 45.90</td>
<td>96.82 ± 42.73</td>
</tr>
<tr>
<td>30</td>
<td>167.27 ± 95.01</td>
<td>143.64 ± 125.45</td>
<td>284.55 ± 93.18</td>
<td>249.54 ± 86.81</td>
<td>165.90 ± 106.82</td>
<td>127.73 ± 86.36</td>
</tr>
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<td>45</td>
<td>194.54 ± 153.63</td>
<td>235.10 ± 157.27</td>
<td>675.45 ± 162.27</td>
<td>312.27 ± 129.09</td>
<td>212.73 ± 144.54</td>
<td>164.09 ± 159.54</td>
</tr>
<tr>
<td>60</td>
<td>135.02 ± 89.09</td>
<td>144.09 ± 118.63</td>
<td>897.28 ± 220.90</td>
<td>523.63 ± 128.18**</td>
<td>255.90 ± 175.45</td>
<td>185.90 ± 116.36</td>
</tr>
<tr>
<td>90</td>
<td>92.73 ± 62.72</td>
<td>85.45 ± 85.05</td>
<td>512.72 ± 260.05</td>
<td>336.81 ± 126.80</td>
<td>183.64 ± 146.36</td>
<td>140.05 ± 100.90</td>
</tr>
<tr>
<td>120</td>
<td>50.10 ± 43.18</td>
<td>41.36 ± 39.54</td>
<td>231.36 ± 136.81</td>
<td>124.09 ± 77.73</td>
<td>110.91 ± 93.18</td>
<td>74.55 ± 37.27</td>
</tr>
</tbody>
</table>

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**Units**: pmol/l (mean ± SD). *P < 0.01 vs females. **P < 0.005 vs females.

EOSDAT: Early onset senile dementia of the Alzheimer type.

LOSDAT: Late onset senile dementia of the Alzheimer type.
concentrations (Fig. 1). In the EOSDAT responders, the rise of plasma GH levels after GHRH injection showed a great individual variability (range = 250–1307 pmol/l). This response was higher in females (897.28 ± 220.90 pmol/l) than in males (523.63 ± 128.18 pmol/l, $P < 0.005$) (Table 1). No sex differences were evident in the other two groups. Significant differences in the basal plasma GH concentrations among the three groups were not detected (Table 1).

GHRH did not significantly modify cardiovascular constants, although systolic pressure changed from 116 ± 16 to 107.5 ± 8.9 mmHg (55–10 min) and diastolic pressure from 75 ± 10.1 to 71.3 ± 6.6 mmHg (5–45 min). The mean heart rate increased from 66.4 ± 3.6 to 69.9 ± 3.2 pulses/min ($P < 0.05$). Other electrocardiographic parameters remained stable. Flushings was observed in 10% of the subjects.

The electroencephalogram (EEG) was abnormal in 95% of SDAT patients owing to the advanced stage of the disease (Table 2). Sequential EEG analysis revealed a fall in frequency (8.75 ± 0.25 Hz, $P < 0.005$) and an increase in amplitude (55.60 ± 6.40 µV, $P < 0.02$) in control subjects and in SDAT patients (7.05 ± 0.90 Hz, $P < 0.05$; 52.5 ± 9.89 µV, $P < 0.005$) from 15 to 45 min after injection, preceding the maximum plasma GH peak.

There was a good correlation of scores between the DRS and MMS tests (Table 3). An inverse curvilinear relationship between the variables mental performance (x) and maximum GH response to GHRH at 60 min (y) was observed ($r = -0.83$). A careful evaluation of the BCRS showed that the axes most significantly affected by GHRH were Axis 4 (orientation, 30%), Axis 7 (psychomotor, 50%), and Axis 8 (mood and behaviour, 40%). According to the MNS, appetite increased in 45% of the patients and in 30% of the controls (2 patients developed a bulimic syndrome), and social interaction improved in 40% of the patients. All these behaviours were transient (33–12 h), whereafter the patients returned to their preliminary FAST stage or even worsened.

### Discussion

According to our results, GHRH can induce a significant increase in the plasma GH levels 60 min after injection in EOSDAT. This GH response is about 15 min delayed with respect to the small peak observed in control subjects. It was suggested that the reduced response of GH to GHRH in aging may be due to increased release of or enhanced sensitivity to somatostatin (Sonntag et al. 1983) as well as to diminished pituitary cAMP response to GHRH (Ceda et al. 1986). In EOSDAT patients characterized by a severe deficit of somatostatin in the neocortex and hippocampus (Davies et al. 1980; Rossor et al. 1984;...
Beal et al. 1986) there is an enhanced response of GH to GHRH. This finding suggests that some further dysfunction concerning somatostatin and GHRH regulation must occur in SDAT. The involvement of impaired monoaminergic systems and inhibitory amino acids in this abnormal response of GH cannot be ruled out (Cababelos et al. 1986a, 1987b). Cholinergic and catecholaminergic dysregulations at the hypothalamic level may influence the effect of GHRH and somatostatin on GH release, and may even account for keeping the basal plasma GH levels abnormally high, in some cases, as reported by Christie et al. (1986). In our subjects, the EOSDAT group had a slightly higher plasma GH concentration than the other two groups, but differences were no longer significant (Table 1). The greater GH response observed in females with EOSDAT may be related to the severity of the disease (stages VI–VII) rather than to real sex differences. Since the majority of our patients were in advanced SDAT stages, these data must be interpreted with caution. However, if the present results can be replicated in early stages of the pathological process underlying mental deterioration, the GHRH test might constitute a useful antemortem marker to differentiate SDAT from other nosological entities with symptoms of dementia.

Our EEG records agree with most data currently reported (McIntyre 1985; Fenton 1986). The effects of GHRH on brain bioelectrical activity in humans partially agree with data reported by Guillemin et al. (1984), Ehlers et al. (1986), and Nistico et al. (1987). However, it appears paradoxical that GHRH, which apparently potentiates slow cortical activity when centrally administered, can induce an increase of psychomotor functions in humans and animals after peripheral injection (Cababelos et al. 1986a, 1987a,b,c). This point needs to be more fully clarified before any conclusion can be reached. Similarly, how GHRH exerts its central effects when injected peripherally is another matter which remains to be elucidated. In rats, ip GHRH increases locomotor activity, but this effect is 3–4 times lower than that elicited by equimolar concentrations of i.c.v. GHRH (Cacabelos et al. 1987a,c). This might be an indirect evidence that GHRH can partially enter the blood-brain barrier (BBB) (Banks & Kastin 1985) or that its central effects after peripheral administration are mediated through a mechanism arising from exposed areas out of the BBB (e.g. the median eminence, hypophysis) (Meisenberg & Simmons 1983) or depend upon GH and/or IGF feedback mechanisms. If this is true, a cautious administration of GHRH might be of use as a palliative therapy in alleviating some impaired psychomotor functions in SDAT if results similar to ours can be obtained in double-blind clinical trials with a larger sample of patients using reliable evaluation procedures.

Summarizing, in EOSDAT patients, GHRH in-

![Graph](image_url)

Fig. 2.
Correlation between the GHRH-induced GH response 60 min after injection and the mental performance of patients with early onset senile dementia of the Alzheimer type 24 h prior to testing.
duces a clear GH response which slightly correlates with the severity of the disease and which is absent in controls and LOSDAT patients. This response is accompanied by EEG and behavioural changes mainly circumscribed to psychomotor functions. In consequence, it seems likely that severe deficits of brain somatostatin might in part be responsible for the enhancement of the GHRH-induced GH response. Finally, if GH differentially responds to GHRH in early stages of the disease, the GHRH test might be useful as an antemortem marker for SDAT.

References


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