
Progressive supranuclear palsy (PSP) has been associated with degenerative changes in cholinergic and dopaminergic neurons in several brain regions. Since acetylcholine is colocalized with the neuropeptide galanin in certain neuronal populations, we measured the concentration of this neuropeptide and neuropeptide Y in cerebrospinal fluid (CSF) of 11 patients with PSP and in 16 age-matched healthy controls. No significant alterations in the CSF levels of galanin or neuropeptide Y were found.

Galanin is a 29-amino acid neuropeptide widely distributed throughout the mammalian and human central nervous system (1-3). In rats, lesions in the medial septal area decrease hippocampal acetylcholine and galanin levels (4). In human basal forebrain, galanin has been found within cholinergic neurons as well as in interneurons (5) including those that appear to degenerate in progressive supranuclear palsy (PSP), Parkinson's disease (PD) and Alzheimer's disease (AD) (6-8). Galanin was found to be increased in surviving basal nucleus of Meynert neurons of Alzheimer's and Parkinson's disease patients, despite significant reductions in choline acetyltransferase activity (9-11). Since galanin prevents acetylcholinesterase release from hippocampus (12), it has been proposed that this neuropeptide can functionally modulate cholinergic related behaviors (13).

Degeneration of cholinergic neurons has also been described in PSP, particularly in caudate-putamen, forebrain, and several brainstem nuclei (6, 14, 15). In addition, CSF acetylcholinesterase activity is significantly reduced in this disorder (16). In an attempt to determine whether galanin would be differentially affected in this patient population (as its colocalization with acetylcholine is only known to occur in the basal forebrain which is less involved in PSP than in AD or PD patients) and to possibly develop neurochemical markers to assist in the ante-mortem diagnosis of PSP, we compared galanin levels in the CSF of patients with PSP to that from normal controls. Neuropeptide Y levels were also measured to contrast with galanin levels in view of the presence of this neuropeptide in striatal areas affected by PSP.

**Material and methods**

**Patient selection**

CSF from 11 progressive supranuclear palsy patients (Mean ± SD, age, 64 ± 6 years, symptom duration, 35 ± 13 months, Columbia Rating Scale, 11 ± 4) was compared to 16 healthy controls (age, 64 ± 14 years). The diagnosis of PSP was based on the presence of all the following features (17): onset after age 50; parkinsonian signs including postural or gait disorder, bradykinesia and/or axial rigidity, without tremor at rest; pseudobulbar signs including dysarthria and dysphagia; extraocular movement abnormalities characterized by supranuclear vertical or out horizontal palsy; progressive course; and no radiologic abnormalities on CT and MRI except subcortical and/or midbrain atrophy.

**Assay procedures**

Thirty ml of CSF were collected by lumbar puncture after overnight fasting at approximately 8.30 am,
separated in 1 ml aliquots and immediately frozen on dry ice and kept at \(-70^\circ C\) until assayed. Galanin was measured by RIA (18, 19). Frozen CSF was lyophilized to dryness and reconstituted with 0.22 ml of assay buffer. Duplicate determinations were performed on each sample by adding 0.1 ml aliquots of sample to polystyrene test tubes containing 0.1 ml of antiserum (recognizes the C-terminal region of galanin and does not cross-react with any other known neuropeptide). Mixtures were incubated 48 h at 4°C and 20,000 cpm of tracer was added in 0.1 ml of assay buffer. The incubation was continued for another 24 h. One ml of assay buffer was then added to each test tube. After vortexing, a goat antirabbit second antibody was added and precipitates were allowed to form for 2 h at room temperature. Tubes were centrifugated and the supernatant aspirated. The radioactivity of pellets was determined in a Beckmann 5000 gamma counter. Repeated determinations on multiple samples revealed an intra- and interassay coefficient of variations of 5 and 12% respectively. Neuropeptide Y was also measured by RIA (20). No rostrocaudal CSF gradient has been previously reported in either of these neuropeptides suggesting a widespread brain and spinal cord contribution (18).

Statistical analysis

Values are reported as the means ± SEM. Statistical analysis for differences in CSF levels of galanin and neuropeptide Y utilized the unpaired two tail t-Test. Correlations were computed between CSF levels and tests of dementia including subtests of attention (Mattis Dementia Rating Scale), memory (Weschler Memory Scale tests) and motor function (Columbia Rating Scale). The Bonferroni correction was used to adjust for the number of correlations and therefore the required significance level was \(p < 0.005\).

Results

CSF galanin levels in PSP patients averaged 15% below those of control subjects, a difference that did not quite attain statistical significance (5.1 ± 0.4 and 6.0 ± 0.3 pg/ml, respectively, \(p < 0.08\), Fig. 1). However, 9 of the 11 PSP patients had galanin levels below the mean of the controls. If the one PSP patient whose galanin value exceeded those of all the controls was excluded from the analysis, a significant difference between groups would be obtained.

CSF galanin levels did not correlate with measures of memory, global dementia or motor scores. There was no correlation between galanin levels and AChE activity in CSF.

Neuropeptide Y concentrations in CSF did not differ from control levels (109 ± 4 and 113 ± 4 pg/ml for PSP and controls, respectively).

Discussion

The finding of similar levels of galanin in the CSF of PSP patients and controls is comparable to the galanin immunoreactivity in CSF of AD patients which was also reported to be no different than age and sex-matched controls (19). In AD, normal CSF levels of galanin seem to reflect the preserved content of galanin in cortical regions, but not the increased levels of this neuropeptide in the basal forebrain (10).

Galanin is known to coexist with acetylcholine only in medial septal neurons, the nucleus basalis, and diagonal band complex. Recent studies have shown that galanin inhibits the release of acetylcholine in the hippocampus (12, 21) and impairs learning (22). Since galanin levels are reportedly normal or even elevated in the brains of patients with Parkinson’s and Alzheimer’s disease, it has been suggested that preserved or even increased galanin may exacerbate the cholinergic and cognitive deficits that accompany the dementia found in these disorders by increased inhibition of the residual cholinergic neurons (9–13). The less severe cognitive impairment
found in PSP patients compared to other dementias is probably related to a relatively preserved hippocampus and cerebral cortex in this disorder compared to the marked cell loss observed in AD brains. On the other hand, the low but preserved concentration of galanin in PSP might contribute to their memory deficits since galanin may inhibit cholinergic neurotransmission.

No alteration in CSF neuropeptide Y levels could be documented in these PSP patients. Neuropeptide Y was also found unchanged in AD patients (23). We were thus unable to confirm a preliminary report of a modest reduction of both neuropeptides in PSP patients (24). If galanin and neuropeptide Y are reduced in the brains of PSP patients, this change must be too small to be reliably detected in samples of lumbar CSF. Therefore, these CSF markers do not appear to have a premortem diagnostic value in PSP.

References