

# Brain amino acids and glutathione in progressive supranuclear palsy

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**Article abstract**—We measured amino acid contents in autopsied brains of seven patients with progressive supranuclear palsy (PSP) and in control subjects (dying without brain disease). Glutathione was also quantitated in rapidly frozen brains of PSP patients, Parkinson's disease (PD) patients, and controls. In PSP, we found glutamic acid markedly increased in the nucleus accumbens; taurine significantly increased in nucleus accumbens, substantia nigra, and globus pallidus; and  $\gamma$ -aminobutyric acid significantly increased in nucleus accumbens and putamen. Glycerophosphoethanolamine contents were significantly increased in most regions. Glutathione, which is significantly decreased in substantia nigra in PD, was increased in this brain region in PSP, suggesting that different mechanisms may be responsible for destruction of dopaminergic nigrostriatal neurons in these two disorders.

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Progressive supranuclear palsy (PSP) is an uncommon degenerative neurologic disorder of unknown etiology first defined as a specific clinicopathologic entity in 1964 by Steele et al.<sup>1</sup> Symptoms resemble those of idiopathic Parkinson's disease (PD), and PSP is said to account for about 4% of patients with parkinsonian symptoms.<sup>2</sup> Patients with PD and PSP both commonly display bradykinesia, axial rigidity, and postural instability, but tremor is unusual in PSP, while vertical gaze paresis and pseudobulbar palsy are common in fully developed PSP. Depression and eventual dementia are frequently seen in PSP. Dopamine agonists and cholinergic antagonists are much less effective in PSP patients than they are in the therapy of PD.<sup>3</sup>

Two recent studies by Kish et al.<sup>4</sup> and Ruberg et al.<sup>5</sup> have begun to uncover the biochemical abnormalities which occur in brain in PSP. Both studies found dopamine and homovanillic acid contents markedly reduced in caudate nucleus and putamen, but normal in nucleus accumbens and cerebral cortex, indicating damage to the nigrostriatal dopaminergic system like that found in PD, but not to the mesolimbic or mesocortical dopaminergic tracts. One study<sup>5</sup> also found a decrease in choline acetyltransferase enzyme activity and in [<sup>3</sup>H]-spiperone binding to cholinergic neurons in many brain regions, including striatum, cerebral cortex, and substantia innominata. Little is known about contents of amino acid neurotransmitters in brain in PSP.  $\gamma$ -Aminobutyric acid (GABA) and aspartic acid contents were normal in autopsied brain, while glutamic acid contents were elevated in many brain regions in three of five PSP patients.<sup>4</sup>

We report measurements of a number of amino acids

and related compounds, including glutathione, in the autopsied brains of seven PSP patients, and we compare these with contents found in control subjects and in patients with PD.

**Patients and control subjects.** Frozen brain tissue was obtained at autopsy from seven patients who had the classic neuropathologic findings of PSP. In each brain, marked cell loss, gliosis, and neurofibrillary degeneration occurred in the substantia nigra, globus pallidus, and subthalamic nucleus, with similar more moderate changes in the striatum, brainstem tegmentum, hypothalamus, and superior and inferior colliculi. The neurofibrillary tangles had a distinctive appearance, with numerous bundles of straight neurofilaments 12 to 16 nm in diameter. Detailed clinical histories were available for five of the seven PSP patients. These five patients suffered from postural and gait disturbances, bradykinesia, rigidity, and paralysis of vertical gaze, and four of them had dementia. None had tremor. Five of the seven PSP patients had been treated with L-dopa and carbidopa with little or no beneficial response.

We obtained frozen brain tissue at autopsy from 42 control subjects without neurologic or psychiatric disease for comparison of amino acid contents with the PSP patients. The mean ages ( $\pm$  SEM) of the controls and PSP patients were  $60.1 \pm 2.4$  and  $71.7 \pm 2.9$  years ( $p < 0.05$ ).

A separate group of 79 control subjects was used for comparison of brain glutathione contents with the PSP patients. These controls included both individuals who had died without brain disease and patients who had died from several brain disorders not known to involve any disorder of glutathione metabolism (including Huntington's disease, cerebellar ataxias, amyotrophic lateral sclerosis, Alzheimer's disease, and schizophrenia). This second group of controls had a mean age of  $58.4 \pm 1.7$  years. Finally, brain glutathione contents in the PSP patients

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**Table 1. Contents of glycerophosphoethanolamine, taurine, glutamate, glutamine, and GABA in various brain regions in progressive supranuclear palsy**

Subjects	Frontal cortex	Occipital cortex	Cerebellar cortex	Caudate nucleus	Putamen	Substantia nigra	Nucleus accumbens	Globus pallidus	Thalamus, medial-dorsal	Dentate nucleus
<b>Glycerophosphoethanolamine</b>										
Controls	0.89 ± 0.06 (29)	0.77 ± 0.07 (27)	1.00 ± 0.08 (26)	0.97 ± 0.07 (33)	0.90 ± 0.07 (19)	1.34 ± 0.08 (33)	0.87 ± 0.13 (16)	1.12 ± 0.13 (16)	0.95 ± 0.09 (21)	1.94 ± 0.48 (19)
PSP	1.15 ± 0.09 (7)	1.10 ± 0.11 (7)	1.43 ± 0.16† (7)	1.30 ± 0.10‡ (7)	1.28 ± 0.19† (7)	1.79 ± 0.22‡ (6)	1.48 ± 0.16‡ (7)	1.61 ± 0.14‡ (7)	1.51 ± 0.12‡ (7)	2.56 ± 0.16 (7)
<b>Taurine</b>										
Controls	1.03 ± 0.07 (30)	0.93 ± 0.07 (28)	2.85 ± 0.20 (26)	1.25 ± 0.08 (33)	1.30 ± 0.09 (19)	1.05 ± 0.06 (33)	0.85 ± 0.08 (16)	1.27 ± 0.07 (16)	0.87 ± 0.06 (21)	1.14 ± 0.10 (18)
PSP	0.98 ± 0.07 (7)	1.05 ± 0.08 (7)	2.25 ± 0.20 (7)	1.44 ± 0.15 (7)	1.43 ± 0.10 (7)	1.48 ± 0.15‡ (6)	1.26 ± 0.09† (7)	1.54 ± 0.11‡ (7)	1.03 ± 0.09 (7)	1.43 ± 0.14 (7)
<b>Aspartic Acid</b>										
Controls	1.14 ± 0.13 (7)	1.65 ± 0.25 (5)	0.84 ± 0.12 (4)	0.86 ± 0.14 (5)	1.06 ± 0.09 (2)	1.43 ± 0.23 (7)	1.08 ± 0.55 (2)	1.80 (1)	1.85 ± 0.29 (2)	0.83 ± 0.26 (3)
PSP	1.22 ± 0.07 (4)	2.08 ± 0.31 (4)	1.24 ± 0.11 (4)	1.10 ± 0.16 (4)	1.43 ± 0.18 (4)	1.24 ± 0.21 (4)	1.41 ± 0.19 (4)	1.09 ± 0.10 (4)	1.32 ± 0.17 (4)	0.98 ± 0.12 (4)
<b>Glutamic Acid</b>										
Controls	8.28 ± 0.28 (31)	8.31 ± 0.23 (28)	9.05 ± 0.32 (26)	10.27 ± 0.31 (33)	11.29 ± 0.56 (18)	5.51 ± 0.24 (33)	6.31 ± 0.35 (16)	5.91 ± 0.47 (16)	9.11 ± 0.42 (19)	4.77 ± 0.35 (18)
PSP	9.32 ± 0.60 (7)	9.58 ± 1.17 (7)	9.60 ± 1.11 (7)	11.71 ± 1.23 (7)	13.02 ± 1.68 (7)	6.13 ± 0.93 (6)	12.14 ± 1.46* (7)	6.25 ± 1.03 (7)	9.04 ± 0.86 (7)	6.30 ± 0.96 (7)
<b>Glutamine</b>										
Controls	4.64 ± 0.29 (27)	4.98 ± 0.56 (24)	5.81 ± 0.34 (22)	4.46 ± 0.11 (29)	4.04 ± 0.44 (18)	4.11 ± 0.35 (27)	4.31 ± 0.44 (19)	5.97 ± 0.56 (16)	4.27 ± 0.34 (17)	4.27 ± 0.32 (14)
PSP	5.71 ± 1.00 (7)	6.31 ± 1.11 (7)	7.61 ± 1.36 (7)	6.57 ± 1.26 (7)	7.25 ± 1.39† (7)	5.85 ± 0.85‡ (7)	7.52 ± 1.30† (7)	7.14 ± 0.98 (7)	5.81 ± 1.11 (7)	5.98 ± 1.00 (7)
<b>GABA</b>										
Controls	1.64 ± 0.08 (31)	1.80 ± 0.10 (28)	1.65 ± 0.08 (26)	2.87 ± 0.15 (33)	2.94 ± 0.19 (19)	6.00 ± 1.22 (33)	4.11 ± 0.29 (16)	7.32 ± 0.40 (16)	1.94 ± 0.16 (21)	4.77 ± 0.21 (18)
PSP	1.23 ± 0.07† (7)	1.43 ± 0.20 (7)	1.40 ± 0.18 (7)	3.58 ± 0.46 (7)	4.37 ± 0.67† (7)	5.06 ± 1.00 (7)	6.45 ± 0.57* (7)	7.21 ± 0.67 (7)	1.33 ± 0.13‡ (7)	4.29 ± 0.71 (7)

\*  $p < 0.001$ .†  $p < 0.01$ .‡  $p < 0.05$ .Values (mean ± SEM) expressed in  $\mu\text{mol/g}$  wet weight, with numbers of subjects in parentheses.

were also compared with those in the brains of 17 patients with neuropathologically confirmed PD (mean age, 73.9 ± 3.2 years).

**Methods.** Brain was frozen at various intervals (range, 1 to 30 hours) after death and stored at  $-70^{\circ}\text{C}$ . Brains were partially thawed to about  $-10^{\circ}\text{C}$  for regional dissection, and specimens were homogenized and deproteinized in 0.4 M perchloric acid prior to amino acid analysis.<sup>8-9</sup> Free amino acids and other Ninhydrin-positive compounds were then quantitated on a Technicon automatic amino acid analyzer, using a single cation exchange column and a lithium citrate buffer elution system.<sup>9</sup> We calculated the significance of differences in mean values for amino compounds between the control and PSP groups with Student's two-tailed  $t$  test.

Using these techniques, we found that contents of eight amino compounds (glycerophosphoethanolamine, taurine, phosphoethanolamine, glutamic acid, glutamine, cystathionine, homocarnosine, and  $\gamma$ -aminobutyryl-lysine) remain unchanged between instantly frozen biopsied human brain and the same biopsy specimens maintained for 48 hours under simulated mortuary conditions.<sup>7</sup> GABA contents, however, rise rapidly after brain death to reach maximal and stable levels within 1 to 2 hours,<sup>7</sup> while aspartate contents are stable for the first 4 hours after brain death but thereafter rise rapidly

in unfrozen brain. The mean contents of glycerophosphoethanolamine, taurine, glutamic acid, and glutamine listed in table 1 for controls and PSP patients are probably representative of the levels actually present during life. So are the contents of aspartic acid, which were calculated only from those brains frozen within 4 hours of the patient's death. The mean GABA contents shown for different brain regions in table 1 are undoubtedly higher than they were during life, but they can properly be compared between controls and PSP patients, since death-to-freezing intervals were in all cases greater than 1 hour and not more than 30 hours.

Glutathione contents decrease steadily after death in unfrozen brain due to autolysis of this tripeptide, so values approximating those occurring during life can only be obtained by limiting studies to specimens with short death-to-freezing intervals.<sup>7</sup> For the comparisons of glutathione between the second group of controls, the PSP patients, and patients with PD (table 2), we used only brain specimens which had been frozen within 8 hours of the patient's death. Total glutathione contents were calculated as the sum of reduced glutathione (GSH), 2  $\times$  oxidized glutathione (GSSG), and glutathione-cysteine mixed disulfide (GS-SCy), a compound resulting from glutathione hydrolysis which is not seen in instantly frozen brain biopsies, but which is always present in autopsied human brain.

**Table 2. Total glutathione contents in rapidly frozen autopsied brain**

	Control	PSP	Parkinson's disease
Death-to-freezing interval (hr)	3.9 ± 0.3 (79)	3.4 ± 0.5 (4)	4.06 ± 0.44 (17)
Frontal cortex	1.80 ± 0.06 (61)	2.04 ± 0.24 (4)	1.55 ± 0.10 (17)
Occipital cortex	1.64 ± 0.06 (63)	1.83 ± 0.29 (4)	1.40 ± 0.11 (14)
Cerebellar cortex	2.01 ± 0.09 (57)	2.53 ± 0.46 (4)	1.59 ± 0.17† (10)
Caudate nucleus	2.24 ± 0.08 (62)	2.86 ± 0.38 (4)	1.83 ± 0.20 (12)
Putamen	2.16 ± 0.10 (36)	3.04 ± 0.36* (4)	1.76 ± 0.13 (11)
Substantia nigra	1.60 ± 0.06 (62)	2.45 ± 0.58* (4)	1.22 ± 0.12* (17)

Glutathione contents (mean ± SEM) are expressed in  $\mu\text{mol/g wet weight}$ , and are the total of GSH, 2 × GSSG, plus GS-SG. Number of subjects in parentheses. \* $p < 0.01$ , † $p < 0.05$ , as compared to the mean for controls for the same brain region.

**Results.** Table 1 lists the mean contents of six compounds (glycerophosphoethanolamine, taurine, aspartic acid, glutamic acid, glutamine, and GABA) in 10 brain regions of the PSP patients and of the neurologically normal controls. Glutamic acid content was significantly and markedly elevated in the nucleus accumbens of the PSP patients, while glutamine contents were significantly increased in the putamen, substantia nigra, and nucleus accumbens. Aspartate contents were similar in all brain regions of the relatively small number of PSP patients and control subjects whose brains had been frozen within 4 hours of death. Taurine contents were significantly higher in the substantia nigra, nucleus accumbens, and globus pallidus of the PSP patients. GABA contents were significantly increased in the putamen and nucleus accumbens of the PSP patients. Finally, glycerophosphoethanolamine contents were significantly increased in most brain regions in the PSP patients.<sup>1</sup>

No significant differences between PSP patients and controls were found for four additional compounds whose contents do not change appreciably between living brain and autopsied brain<sup>7</sup>: phosphoethanolamine, cystathionine, homocarnosine, and  $\gamma$ -aminobutyryllysine. In the interests of brevity, values for these compounds are not listed in table 1. One neurotransmitter amino acid, glycine, could not be compared between PSP patients and controls, because its contents increase progressively and markedly after death in unfrozen brain.<sup>7</sup>

Table 2 compares total glutathione contents in six brain regions of the PSP patients with the same brain regions in a group of control subjects and a group of PD patients. For these comparisons, we only used data for brain specimens with death-to-freezing intervals of 8 hours or less, so that glutathione contents found would approximate those present during life. Because insufficient specimens were available from patients dying without brain disease, where tissue had been frozen this rapidly, we included among the controls for glutathione contents patients who died with various brain disorders. None of the latter, however, has been shown to involve any abnormality of glutathione content. Whereas total glutathione contents were significantly low in the substantia nigra and cerebellar cortex of the PD patients as

compared with the control subjects, and were somewhat low in other brain regions of the PD patients, the opposite was true for the PSP patients, with glutathione contents being significantly higher than the controls in the substantia nigra and the putamen.

**Discussion.** Of the four amino acids measured which are known to act as neurotransmitters (glutamate and GABA), or which may act as neurotransmitters or neuromodulators (aspartate and taurine), only aspartate contents were unremarkable in all brain regions in the PSP patients. Mean glutamate content was markedly elevated (twofold) in the nucleus accumbens of PSP patients. Glutamate contents tended to be higher in most brain regions in the PSP patients than in the controls, but they were not significantly elevated in the occipital cortex, striatum, globus pallidus, and dentate nucleus as found by Kish et al.<sup>4</sup> These investigators did not report glutamate content in the nucleus accumbens. We found mean contents of taurine significantly elevated in the nucleus accumbens, globus pallidus, and substantia nigra, and GABA contents significantly increased in the putamen and nucleus accumbens. In PD patients, a significant increase in GABA content has been reported in the putamen.<sup>10</sup> It is possible that loss of dopaminergic input to the striatum in both PD and in PSP causes a change in the physiologic regulation of striatal GABAergic neurons to produce this increased GABA content.

The increased glycerophosphoethanolamine contents which we observed in most brain regions in our PSP patients, as compared with control subjects who died without brain disease, may reflect loss of neuronal cell bodies with relative preservation of myelin in these regions. White matter contains much greater amounts of glycerophosphoethanolamine than does gray matter, and we found similar increases in glycerophosphoethanolamine in the autopsied brains of patients dying with Huntington's disease,<sup>11</sup> presumably secondary to neuronal loss.

It is notable that abnormalities of mean glycerophosphoethanolamine, taurine, glutamate, and GABA contents all occurred in the nucleus accumbens of our PSP patients. Kish et al<sup>4</sup> and Ruberg et al<sup>8</sup> did not find abnormalities of dopamine or homovanillic acid contents in the nucleus accumbens of their PSP patients, although the latter group reported mean choline acetyltransferase activity low in this region. In the future, perhaps more attention should be directed to searching for neuropathologic and neurochemical changes in the nucleus accumbens in PSP.

The decrease in total glutathione content in the substantia nigra in PD has been suggested<sup>12</sup> as possibly indicating consumption of GSH in the detoxification of free radicals, including those derived from dopamine. This finding provides a clue as to what may be causing the progressive loss of dopaminergic nigrostriatal neurons in PD. Because patients with PSP have symptoms resembling those of PD,<sup>2</sup> and because both disorders are biochemically similar in their striatal deficiency of dopamine and homovanillic acid,<sup>4,5</sup> we thought it important to measure glutathione contents in PSP. In a small group of PSP patients (table 2), no such decrease

in total glutathione was found in the substantia nigra, even though three of these four patients were apparently being treated with L-dopa up until their deaths. This observation also suggests that the low nigral glutathione found at death in PD patients is unlikely to be due simply to excessive dopamine formed from the L-dopa used in therapy.

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