Multiple System Atrophy Is Distinguished from Idiopathic Parkinson's Disease by the Arginine Growth Hormone Stimulation Test

Maria Teresa Pellecchia, MD,[1] Katia Longo, MD,[1] Rosario Pivonello, MD,[2] Claudio Lucetti, MD,[3] Roberta Marchese, MD,[4] Annalisa Spampani, MD,[5] Michela Manfredi, MD,[6] Antonio Epifanio, MD,[7] Mariachiara Sensi, MD,[8] Tomaso Scaravilli, MD,[9] Fulvio Bracco, MD,[9] Roberto Eleopra, MD,[8] Letterio Morgante, MD,[7] Edoardo Donati, MD,[6] Roberto Marconi, MD,[5] Giovanni Abbruzzese, MD,[4] Ubaldo Bonuccelli, MD,[3] Mario Zappia, MD,[10] Annamaria Colao, MD,[2] and Paolo Barone, MD[1]

Objective: Multiple system atrophy (MSA) may be difficult to distinguish from idiopathic Parkinson's disease (PD). Our aim was to evaluate the accuracy of the arginine growth hormone (GH) stimulation test in distinguishing between MSA and PD in large populations of patients.

Methods: We measured the GH response to arginine in 69 MSA (43 MSAp [parkinsonism as the main motor feature] and 26 MSAc [cerebellar features predominated]) patients, 35 PD patients, and 90 healthy control subjects. We used receiver-operating curve analysis to establish the arginine cutoff value that best differentiated between MSA and PD.

Results: The GH response to arginine was significantly lower ($p < 0.01$) in MSA than in either PD patients or control subjects. At a cutoff level of 4μg/L, arginine distinguished MSAp from PD with a sensitivity and specificity of 91% and MSAc from PD with a sensitivity of 96% and specificity of 91%. The arginine test had a positive predictive value for MSA of 95%. The GH response to arginine was not affected by disease duration or severity, MSA motor subtype, pyramidal signs, response to dopaminergic therapy, or magnetic resonance imaging findings.

Interpretation: The GH response to arginine differentiates MSA from PD with a high diagnostic accuracy. The results suggest an impairment of cholinergic central systems modulating GH release in MSA.

Ann Neurol 2006;60:611–615

Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder clinically characterized by various combinations of parkinsonian, cerebellar, autonomic, and pyramidal signs.[1] It may be difficult to differentiate MSA from idiopathic Parkinson's disease (PD) because rest tremor, asymmetric akinesia, and rigidity can occur in both conditions.[2] Furthermore, in the early stages of the disease, 30% of MSA patients respond to L-dopa treatment and autonomic dysfunction may not appear.[2] About 25% of cases with pathological evidence of MSA had a clinical diagnosis of PD during their life.[3] It is important to differentiate between MSA and PD because response to therapy and prognosis vary according to the disorder. Although the diagnosis of MSA is largely based on clinical criteria, there is a search for laboratory and instrumental tests that aid to distinguish between MSA and PD. Kimber and colleagues[4] proposed the growth hormone (GH) response to clonidine, an α_2-adrenoceptor agonist that stimulates GH-releasing hormone (GHRH). They found no increase in GH levels after clonidine in patients with MSA, but found a preserved response in patients with PD. However, subsequent studies did not confirm these results, and the capacity of the clonidine test to differentiate between the two disorders is controversial.[5–8] In a pilot study, we recently observed that GH response to arginine, an amino acid that inhibits somatostatin release, was significantly reduced in a

From the Departments of [1]Neurological Sciences and [2]Molecular and Clinical Endocrinology and Oncology, University of Naples "Federico II," Naples; [3]Department of Neurosciences, University of Pisa, Pisa; [4]Department of Neurosciences, Ophthalmology and Genetics, University of Genoa, Genoa; [5]Department of Neurology, Misericordia Hospital, Grosseto; [6]Neurology Unit, Poliambulanza Hospital, Brescia; [7]Department of Neuroscience, Psychiatry and Anesthesiology, University of Messina, Messina; [8]Department of Clinical Neurosciences, S. Anna University Hospital, Ferrara; [9]Department of Neuroscience, University of Padua, Padua; and [10]Department of Neurosciences, University of Catania, Catania, Italy.

Received Feb 28, 2006, and in revised form Jun 12. Accepted for publication Jul 17.

Published online Sep 6, 2006, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20956

Address correspondence to Dr Barone, Department of Neurological Sciences, University Federico II, Via Pansini 5, 80131 Naples, Italy. E-mail: barone@unina.it

small number of MSA patients compared with PD patients.[9] The aim of this study was to evaluate the diagnostic accuracy of the arginine test in differentiating between MSA and PD in a large number of patients.

Patients and Methods

Sixty-nine patients with MSA (43 MSAp and 26 MSAc, diagnosed as indicated below), 35 patients with idiopathic PD, and 90 healthy control subjects (55 men and 35 women; mean age, 60.9 ± 1.5 years) were enrolled in the study. Detailed clinical information was obtained from the patient's history, neurological examination, and hospital records. MSA was diagnosed by movement disorder specialists experienced in parkinsonian disorders according to consensus criteria.[10] All MSA patients met consensus criteria. Patients were diagnosed MSAp when parkinsonism was the main motor feature and with MSAc when cerebellar features predominated. Six MSAp patients were classified as possible and 37 as probable MSA cases. All MSAc patients were classified as probable MSA cases. Secondary causes of autonomic dysfunction were excluded. Idiopathic PD was diagnosed according to UK Parkinson's Disease Brain Bank Diagnostic Criteria.[11] Patients affected by PD had no history of familial parkinsonism or clinical features of autonomic dysfunction. Dopaminergic pathways are involved in the control of GH secretion by releasing GHRH or somatostatin in relation to endogenous dopaminergic tone.[12] To avoid possible interactions, we stopped administration of antiparkinsonian drugs at least 2 weeks before the test, which is the generally accepted washout period in clinical studies.[13] No patient had a history of endocrinological disorders, psychiatric illness, or substance abuse. Parkinsonism was staged according to Hoehn and Yahr.[14]

The study was approved by the local ethics committee. After providing informed consent, patients and control subjects underwent the arginine test in the outpatient clinic after a 12-hour fast. After subjects had rested in a supine position for at least 30 minutes, baseline samples (T0) were collected from a cannulated antecubital vein. Then 30gm arginine (arginine hydrochloride, 30% solution) was infused intravenously over 30 minutes, and blood was sampled every 30 minutes for 1 hour (T30, T60, and T90) thereafter. Blood pressure and heart rate were monitored throughout the test. Blood samples were centrifuged and frozen (−20°C) immediately after sampling. After all subjects had undergone the arginine test, the frozen samples were shipped in a single batch on ice to a central laboratory. Serum GH was measured with a commercially available immunoradiometric kit (Chematil, Angri, Italy). The sensitivity of the assay is 0.15μg/L.

We used the peak serum GH response for comparison purposes in the statistical analysis. The GH response, namely, the increase after the administration of arginine versus baseline value, was evaluated with analysis of variance for repeated measures. We used analysis of variance followed by the Bonferroni test to compare GH peaks among groups. Receiver-operating characteristic analysis was used to establish the cutoff value that resulted in the highest sensitivity and specificity (ie, the highest diagnostic accuracy) in differentiating case groups by the GH response to arginine. Significance was set at 5%. Pearson's correlation (or Spearman's correlation if appropriate) was used to evaluate the correlation between GH peaks and disease variables. In MSA patients, we evaluated differences in GH response to arginine according to motor subtype (MSAp or MSAc), the presence or absence of pyramidal signs, and the presence or absence of typical abnormal findings at magnetic resonance imaging (MRI) using the Mann–Whitney *U* test.

Results

Table 1 shows the demographic and main clinical features of the MSAp, MSAc, and PD patients. Mean age and mean disease duration were similar in the three groups. Hoehn and Yahr stage was significantly higher in MSAp than in PD ($p < 0.01$). The frequency of orthostatic hypotension and urinary incontinence was similar in the two MSA subgroups. Baseline serum GH levels (mean ± standard error [SE]) were similar in PD patients (0.32 ± 0.14μg/L), MSA patients (0.20 ± 0.05μg/L in MSAp; 0.23 ± 0.05μg/L in MSAc), and control subjects (0.5 ± 0.03μg/L). Arginine did not cause any significant changes in blood pressure or heart rate in any group.

As shown in Figure 1, arginine induced an increase in GH concentration in control subjects (mean peak ± SE, 10.1 ± 0.86μg/L) and in patients with PD (8.57 ± 0.85μg/L), but not in patients with MSAp (1.63 ± 0.25μg/L) or MSAc (1.57 ± 0.27μg/L). The GH peak was lower in MSAc and MSAp patients than in PD patients ($p < 0.001$) and control subjects ($p < 0.001$). Receiver-operating characteristic analysis showed that sensitivity and specificity were maximum at a GH concentration of 4μg/L. At this optimum cutoff level, the arginine test distinguished between MSAp and PD patients with a sensitivity and specificity of 91% and between MSAc and PD patients with a sensitivity of 96% and specificity of 91% (Fig 2). Considering the two MSA groups collectively, the sensitivity and specificity of the test were 93 and 91%, respectively, and the positive predictive value was 95%. Table 2 shows the sensitivity and specificity of different cutoff levels.

There was no correlation between disease duration and GH response to arginine in any of the groups studied. Similarly, there was no correlation between Hoehn and Yahr stage and GH response to arginine in MSAp and PD patients. GH response to arginine was blunted in both possible (n = 6; mean ± SE, 1.81 ± 0.43μg/L) and probable MSAp patients (n = 37; mean ± SE, 1.61 ± 0.29μg/L). The GH response of MSA patients was not related to MSA diagnostic category (possible or probable), to the presence or absence of pyramidal signs, or to MRI findings. Finally, the GH response to arginine did not differ between MSAp patients who responded to dopaminergic therapy (mean ± SE, 1.8 ± 0.56μg/L) and those who did not

Table 1. Demographic and Clinical Features of Patients with Multiple System Atrophy and Parkinson's Disease

Characteristics	MSAp (n = 43)	MSAc (n = 26)	PD (n = 35)
Sex: M/F	26/17	11/15	23/12
Mean age ± SE, yr	63.9 ± 0.9	61.5 ± 1.4	60.6 ± 1.6
Mean disease duration ± SE, yr	3.8 ± 0.3	4.2 ± 0.6	3.1 ± 0.4
Hoehn and Yahr stage	2.9 ± 0.1[a]	—	1.9 ± 0.1
Bradykinesia, %	88	69	91
Rigidity, %	95	38	94
Resting or postural tremor, %	34	38	57
Postural instability, %	70	69	14
Pyramidal signs, %	30	42	0
Cerebellar signs, %	16	100	0
Orthostatic hypotension, %	72	69	0
Urinary incontinence, %	93	96	0
Present response to dopaminergic therapy, %	11	–	100
Previous response to dopaminergic therapy, %	28	–	–
MRI findings	PA or HPR in 42% Normal in 46% ND in 12%	CA or HC in 70% Normal in 15% ND in 15%	Normal in 80% ND in 20%

[a] $p < 0.01$ vs Parkinson's disease (PD).

MSAp = parkinsonian-type multiple system atrophy; MSAc = cerebellar-type multiple system atrophy; SE = standard error; MRI = magnetic resonance imaging; PA = putaminal atrophy; HPR = hyperintense putaminal rims; CA = cerebellar atrophy; HC = "hot cross bun" sign; ND = not done.

(mean ± SE, 1.67 ± 0.29). The GH response to arginine was not correlated with responsiveness to dopaminergic therapy.

Discussion

The difficulty in differentiating MSA from PD is reflected by the number of investigations used to support the clinical diagnosis. Autonomic function tests, sphincter electromyography, routine and diffusion-weighted MRI, and functional imaging with positron emission tomography and single-photon emission computed tomography are the investigations most frequently used in the diagnosis of MSA. However, these tests may be limited in their accuracy (where known) and are not always available. The results of our study demonstrate that the GH response to arginine is highly sensitive and specific in distinguishing MSA from PD. A reduced GH response to arginine predicted MSA with an accuracy of 95%. To our knowledge, a similarly high diagnostic accuracy in large series of patients affected by MSA and PD has been obtained by only sympathetic cardiac neuron scintigraphy with 123-metaiodobenzylguanidine.[15]

GH secretion from the pituitary gland is stimulated by GHRH and inhibited by somatostatin.[12,16] GHRH and somatostatin are hypothalamic neurohormones, the pulsatile secretion of which is modulated by various neuronal networks, especially the noradrenergic and

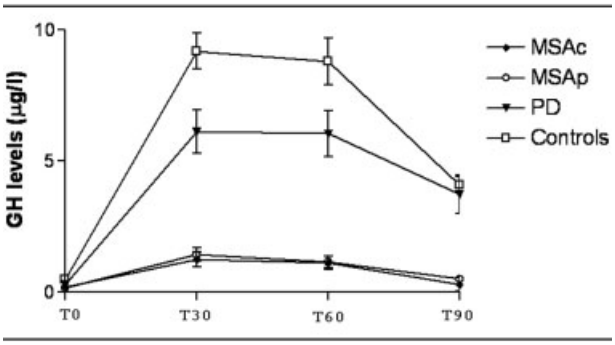

Fig 1. Growth hormone response (mean ± standard error) to the arginine test in patients with multiple system atrophy with parkinsonism as the main motor feature (MSAp; circles*), multiple system atrophy with predominantly cerebellar features (MSAc;* diamonds*), or Parkinson's disease (PD; triangles) and in control subjects* (squares).

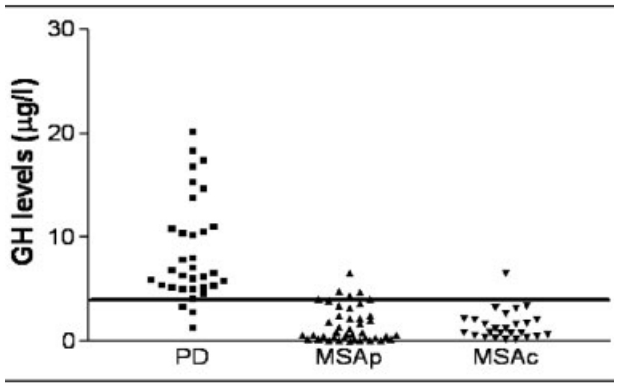

Fig 2. Distributions of growth hormone (GH) peaks after the arginine test in patients with Parkinson's disease (PD), multiple system atrophy with parkinsonism as the main motor feature (MSAp), or multiple system atrophy with predominantly cerebellar features (MSAc). The continuous horizontal line *indicates the optimum cutoff value of 4µg/L.*

Table 2. Sensitivity, Specificity, and Positive Predictive Value (%) of the Arginine Test at Different Cutoff Levels

Cutoff	2.1μg/L	3.25μg/L	4μg/L[a]	5.1μg/L	6.7μg/L
MSAp vs PD, %					
Sensitivity	68	80	91	98	100
Specificity	97	94	91	79	48
Positive predictive value	97	94	93	85	71
MSAc vs PD, %					
Sensitivity	77	88	96	96	100
Specificity	97	94	91	79	48
Positive predictive value	95	92	89	78	60

[a]Maximum sum of sensitivity and specificity.

MSA = multiple system atrophy; PD = Parkinson's disease.

cholinergic systems.[12,16] Activation of hypothalamic α_2-adrenoceptors and muscarinic cholinergic receptors (probably by stimulation of GHRH and inhibition of somatostatin release, respectively) induces GH release.[12,16] Therefore, both the α_2-adrenoceptor agonist clonidine and arginine, which probably activates the cholinergic system, have been used to evaluate pituitary GH secretion and reserve.[17]

The clonidine test first emerged as a promising tool in the differential diagnosis of PD and MSA in 1997. Kimber and colleagues[4] studied 14 PD patients, 31 MSA (15 MSAp and 16 MSAc) patients, and 27 healthy control subjects. No patient had been taking antiparkinsonian drugs for at least 3 months. After clonidine, GH increased in patients with PD and in control subjects, but not in patients with MSA, in neither the parkinsonian nor cerebellar form. The difference in mean GH between the PD and MSA groups was highly significant.[4] Clarke and coworkers[5] did not find a difference in mean GH response to clonidine among 9 MSA, 8 early untreated PD, and 9 advanced PD patients treated with L-dopa and/or dopamine agonists. Tranchant and investigators[6] studied 19 PD and 7 MSA (5 MSAp, 2 MSAc) patients. All but one patient received long-term treatment with L-dopa and/or dopamine agonists. Dopaminergic drugs were not administered on the morning of the test. In this study, the clonidine test had sensitivity of 100% for MSA diagnosis, but a poor specificity (63%). Strijks and coworkers[7] performed the clonidine test in 21 early idiopathic PD and 11 MSAp patients. Antiparkinsonian drugs were discontinued 12 hours before the test. Patients affected by PD had a significantly higher GH response to clonidine than did MSA patients, but the frequency of patients with normal and altered GH response to clonidine did not differ between the two groups. The sensitivity of the test for MSA was 73%, whereas its specificity was 57%.[7] The largest study of the clonidine test conducted so far included 21 PD, 23 MSAp, and 22 MSAc patients.[8] Antiparkinsonian medication was stopped 48 hours before the test. The clonidine test had a sensitivity of 78% for the diagnosis of MSAp and 63% for the diagnosis of MSAc with a specificity of 86% versus PD. The discrepancies among results of the clonidine test are probably related to different washout periods from dopaminergic drugs, different pharmacological treatments, and the small number of patients enrolled.

According to the results of a pilot study in 12 MSA and 10 PD patients, the arginine test appeared to be more accurate in differentiating between MSA and PD than the clonidine test.[9] Here, we show in a much larger number of patients that the GH response to arginine is impaired in MSA, but not in PD. There is a body of evidence implicating the intrahypothalamic cholinergic pathways in the impaired GH response to arginine. Modulation of GH release by an intrahypothalamic cholinergic pathway has been demonstrated in animals and humans.[18,19] An intrahypothalamic cholinergic defect in MSA was suggested by autopsy and clinical findings.[20,21] Acetylcholine-transferase activity, a marker of acetylcholine-containing neurons, was found to be greatly reduced in the hypothalamus of deceased MSA patients.[20] Lastly, the finding of an altered vasopressin response to cholinomimetic agents in MSA patients indicates impairment of intrahypothalamic cholinergic neurons.[21] However, we cannot exclude that the lack of GH response to arginine in MSA patients is related to extrinsic cholinergic pathways. In fact, brainstem cholinergic nuclei projecting to the hypothalamus, namely, the pedunculopontine and laterodorsal tegmental nuclei, are impaired in MSA.[22] Whatever the mechanism, the fact that an abnormal GH response to arginine in MSA occurred regardless of motor subtype, disease severity, or clinical features suggests that cholinergic central systems that modulate GH release are specifically affected in MSA. This would account for the decreased GH response to arginine in MSA and not in PD.

The lack of a pathological diagnosis of MSA and PD is a limitation of our study. We used clinical criteria as the gold standard to evaluate whether the GH response to arginine differed between clinically defined MSA and PD. Based on our results, a prospective study in

early parkinsonism is warranted to assess the predictive value of the arginine test. Interestingly, in our MSAp population, all six patients with a diagnosis of possible MSA had an altered GH response on enrollment in the study. Two years later, these patients met diagnostic criteria for probable MSA (data not shown).

In conclusion, we show that GH response to arginine did not differ in relation to disease duration or severity, MSA motor subtype, corticospinal involvement, or response to dopaminergic therapy. Moreover, GH response to arginine was significantly reduced in MSA patients regardless of MRI findings. Taken together, these results suggest that a reduced GH response to arginine is a sensitive marker of MSA, and that the arginine test may be useful in the early diagnosis of MSA. The safety and low cost of the arginine test may be further reasons to support its use in clinical practice.

This work was supported by the Italian Ministry of Health (Ricerca Finalizzata, IRCCS-Don Gnocchi 2003, P.B.).

We thank J. Gilder for text editing.

References

1. Quinn N. Multiple system atrophy. In: Marsden CD, Fahn S, eds. Movement disorders III. London: Butterworth, 1994: 262–281.
2. Wenning GK, Colosimo C, Geser F, Poewe W. Multiple system atrophy. Lancet 2004;3:93–103.
3. Colosimo C, Albanese A, Hughes AJ, et al. Some specific features differentiate MSA (striatonigral degeneration) from IPD. Arch Neurol 1995;52:294–298.
4. Kimber JR, Watson L, Mathias CG. Distinction of idiopathic Parkinson's disease from multiple system atrophy by stimulation of growth-hormone release with clonidine. Lancet 1997; 349:1877–1881.
5. Clarke CR, Ray PS, Speller JM. Failure of the clonidine growth-hormone stimulation test to differentiate multiple-system atrophy from early or advanced idiopathic Parkinson's disease. Lancet 1999;353:1329–1330.
6. Tranchant C, Guiraud-Chaumeil C, Echaniz-Laguna A, et al. Is clonidine growth hormone stimulation a good test to differentiate multiple system atrophy from idiopathic Parkinson's disease? J Neurol 2000;415:853–856.
7. Strijks E, van't Hof M, Sweep F, et al. Stimulation of growth-hormone release with clonidine does not distinguish individual cases of idiopathic Parkinson's disease from those with striatonigral degeneration. J Neurol 2002;249:1206–1210.
8. Lee EAH, Joon KB, Lee WY. Diagnosing multiple system atrophy with greater accuracy: combined analysis of the clonidine-growth hormone test and external anal sphincter electromyography. Mov Disord 2002;17:1242–1247.
9. Pellecchia MT, Pivonello R, Salvatore E, et al. Growth hormone response to arginine test distinguishes multiple system atrophy from Parkinson's disease and idiopathic late-onset cerebellar ataxia. Clin Endocrinol 2005;62:428–433.
10. Gilman S, Low PA, Quinn N, et al. Consensus statement on the diagnosis of multiple system atrophy. J Auton Nerv Syst 1998;74:189–192.
11. Gibb WR, Lees AJ. The relevance of the Lewy body in the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745–752.
12. Muller EE, Locatelli V, Cocchi D. Neuroendocrine control of growth hormone secretion. Physiol Rev 1999;79:511–607.
13. The Parkinson Study Group. Levodopa and the progression of Parkinson's disease. N Engl J Med 2004;351:2498–2508.
14. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967;17:427–442.
15. Braune S. The role of cardiac metaiodobenzylguanidine uptake in the differential diagnosis of parkinsonian syndromes. Clin Auton Res 2001;11:351–355.
16. Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 1998;19:717–797.
17. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency:summary statement of the Growth Hormone Research Society workshop on adult growth hormone deficiency. J Clin Endocrinol Metab 1998;83:379–381.
18. Tago H, McGeer PL, Bruce G, et al. Distribution of choline acetyltransferase-containing neurons of the hypothalamus. Brain Res 1987;415:49–62.
19. Caffè AR. Architecture of the mammalian pituitary cholinergic system with observations on a putative blood acetylcholine sensor. Histol Histopathol 1996;11:537–551.
20. Spokes EGS, Bannister R, Oppenheimer DR. Multiple system atrophy with autonomic failure. J Neurol Sci 1979;43:59–82.
21. Norbiato G, Bevilacqua M, Righini V, et al. Altered vasopressin response to metoclopramide in multiple system atrophy: evidence of a cholinergic defect in the hypothalmus. Acta Neurol Scand 1992;85:299–301.
22. Benarroch EE, Schmeichel AM, Parisi JE. Depletion of mesopontine cholinergic and sparing of raphe neurons in multiple system atrophy. Neurology 2002;59:944–946.