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ORIGINAL ARTICLE

Growth hormone response to arginine test distinguishes multiple system atrophy from Parkinson's disease and idiopathic late-onset cerebellar ataxia

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Summary

Objective Multiple system atrophy (MSA) is difficult to distinguish from idiopathic Parkinson's disease (PD) and idiopathic late-onset cerebellar ataxia (ILOCA). This study aimed to evaluate GH response to three different GH stimulation tests in order to establish a reliable test to differentiate these degenerative disorders.

Design Twelve patients with MSA, 10 with PD, eight with ILOCA and 30 healthy controls entered the study. They were submitted to clonidine, arginine, and GH-releasing-hormone (GHRH) + arginine tests in a random manner on three different nonconsecutive days. The peak serum GH response was used as a primary variable for analysis of stimulation tests. By ROC analysis, the optimum cut-off level was considered as the cut-off with the maximal sum of sensitivity and specificity.

Results After clonidine administration, GH peak was significantly lower in patients with MSA than in those with ILOCA ($P < 0{\cdot}05$) and in the controls ($P < 0{\cdot}001$). At the optimum cut-off level of 5 mU/l, the clonidine test distinguished patients with MSA from those with PD with a sensitivity and specificity of 78%. Moreover, this test distinguished patients with MSA from those with ILOCA with a sensitivity of 100% and a specificity of 75% at a cut-off level of 5 mU/l, and with a sensitivity of 75% and a specificity of 100% at the cut-off level of 7·6 mU/l. After arginine administration, the GH peak was significantly lower in patients with MSA than in those with ILOCA ($P = 0{\cdot}001$) and in controls ($P < 0{\cdot}001$). At the optimum cut-off level of 5 mU/l, the arginine test distinguished patients with MSA from those with PD with a sensitivity and a specificity of 100%. At a GH peak cut-off value of 3·6 mU/l the arginine test distinguished patients with MSA from those with ILOCA with a sensitivity and specificity of 100%. After GHRH + arginine administration, a significant GH increase was found in all groups of patients and controls.

Conclusions The GH response to arginine administration is impaired in MSA. Therefore, the arginine test showed the highest diagnostic accuracy to distinguish MSA from both PD and ILOCA, and could be used in the clinical practice of these neurodegenerative diseases.

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Introduction

Multiple system atrophy (MSA) is an adulthood-onset sporadic neurodegenerative disorder characterized by parkinsonism, cerebellar ataxia, autonomic failure and pyramidal signs.[1] Two major motor presentations can be distinguished clinically. MSA subtype P (MSA-P) is more frequent (occurs in 80% of cases) and is characterized by parkinsonian features. MSA subtype C (MSA-C) is less frequent (20% of cases) and is characterized by cerebellar ataxia.[1] In the early stages of the disease it is difficult to distinguish MSA-P from idiopathic Parkinson's disease (PD) because of strict clinical similarities.[2] Indeed, a significant percentage of cases initially diagnosed as PD are recognized as MSA during the course of the disease or at postmortem examination.[3] The differential diagnosis between MSA and PD is important, as response to therapy and prognosis vary according to the disorder.

In a previous study,[4] GH response to the clonidine test was described as an accurate test in differentiating MSA from PD: only patients with MSA had no GH response to clonidine, suggesting a dysregulation of the central noradrenergic pathways in this disease. These findings were, however, not confirmed by subsequent studies.[5,6]

Additionally, the early stages of MSA-C are difficult to distinguish from idiopathic late-onset cerebellar ataxia (ILOCA), a group of sporadic degenerative ataxias, whose prognosis is significantly better than that of MSA. At present, no study has evaluated the potential usefulness of GH stimulation tests in differentiating MSA and ILOCA patients. Different stimulation tests are currently used in clinical practice to evaluate GH secretion and reserve.[7] These tests are based on the administration of GH-releasing compounds using different mechanisms and via different nervous pathways.[8]

The aim of the current study was to evaluate GH response to clonidine and arginine tests and to GH-releasing-hormone (GHRH) plus arginine test to differentiate patients with MSA, PD and ILOCA.

Patients and methods

Patients

Twelve patients with MSA (five with MSA-P and five with MSA-C), 10 patients with idiopathic PD, eight patients with ILOCA and 30 healthy controls were enrolled in the study. The diagnosis of MSA was performed according to Quinn's criteria,[1] idiopathic PD according to UK Parkinson's Disease Brain Bank Diagnostic Criteria[9] and ILOCA according to clinical criteria[10]. None of the patients included in the study had been taking antiparkinsonian medication for at least 1 month before the study. All subjects had a normal body mass index and did not have any history of endocrinological disorder or substance abuse. To improve the reliability of the results of the current study, we excluded patients with a recent diagnosis and those at the early stages of disease. Only patients with a clear clinical picture of the different diseases were included. Patients' characteristics are shown in Table 1.

Study protocol

The study protocol was performed on all subjects after their informed consent had been obtained and the study approved by the Local Ethics Committee at the 'Federico II' University of Naples. All subjects were first evaluated at a screening visit for a complete medical history, physical examination and routine laboratory tests. Blood samples were then taken for basal evaluation of pituitary, thyroid, adrenal and gonadal hormones and particularly for the evaluation of basal GH, IGF-I and IGF-binding protein 3 (IGF-BP3) levels. Subsequently, they were submitted to three different GH stimulation tests, performed on three different nonconsecutive days. The three tests were administered in random order on each patient.

Methods

GH stimulation tests. Clonidine, arginine and GHRH + arginine tests were performed on all subjects after inserting a venous catheter 30 min before the initial baseline blood sampling, according to the following procedures:[11]

1) Clonidine test: clonidine (Catapresan, Boehringer, Germany) was given orally at the dose of 300 μg.

Table 1. Clinical characteristics of enrolled patients with multiple system atrophy (MSA), Parkinson's disease (PD), idiopathic late-onset cerebellar ataxia (ILOCA) and controls. The values are expressed as mean ± SE

	Controls ($n = 30$)	MSA ($n = 12$)	PD ($n = 10$)	ILOCA ($n = 8$)
Sex (M/F)	20/10	7/5	7/3	6/2
Age (years)	62·5 ± 0·6	62·2 ± 0·9	62 ± 1·2	61·2 ± 0·7
BMI (kg/m^2)	22·5 ± 0·4	22·5 ± 0·5	22·6 ± 0·6	22·4 ± 0·5
Disease duration (years)	–	4 ± 0·5	3 ± 0·4	7·7 ± 1·3

2) Arginine test: arginine (Arginine Hydrochloride, 10% solution) was infused i.v. over 30 min at the dose of 30 g.

3) GHRH arginine test: 100 μg of GHRH (Geref Diagnostic, Serono, Rome, Italy) was administered i.v. after infusion of arginine over 30 min.

Blood was sampled 30 min after administration of the provocative agents and every 30 min thereafter up to 2 h. During each test, blood samples were centrifuged and frozen (–20 °C). Following completion of all stimulation tests, the frozen samples were transported on ice in a single batch to a central laboratory. As GH deficiency could affect interpretation of results, the GHRH+arginine test was used to exclude this deficiency in subjects taking part in the current study. A GH peak higher than 18 mU/l post testing was considered a normal response, therefore excluding a diagnosis of GH deficiency.[12] Although a GH peak of 6 mU/l is the standard cut-off for distinguishing GH deficiency in clonidine and arginine tests, low diagnostic accuracy has been reported.[12] GHRH + arginine was therefore considered to be the only reliable test for this purpose.

GH, IGF-I and IGF-BP3 measurements

Blood samples were collected at 8 a.m., after a 12-h fasting period, in EDTA containing tubes, rapidly centrifuged, and then stored at –20 °C until used for assay. Serum GH was measured using a commercially available immunoradiometric (IRMA) kit. The sensitivity of the assay was 0·30 mU/l. The inter- and intra-assay coefficients of variation (CV) were 5·1 and 2·6%, respectively. Plasma IGF-I was measured after ethanol extraction using a commercially available IRMA kit. The sensitivity of the assay was 0·8 μg/l. The intra-assay CV were 3·4, 3·0 and 1·5% for the low, medium and high point of the curve, respectively. The inter-assay CVs were 8·2, 1·5 and 3·7% for the low, medium and high point of the curve, respectively. Plasma IGF-BP3 was measured using commercial available IRMA kits. The sensitivity of the assay was 0·5 μg/l. The intra-assay CV were 3·9, 3·2 and 1·8% for the low, medium and high point of the curve, respectively. The inter-assay CV were 0·6, 0·5 and 1·9% for the low, medium and high point of the curve, respectively.

Statistical analysis

Data were expressed as mean ± SE. The peak serum GH response was used as primary variable for analysis of stimulation tests. The GH response, namely the GH increase after the administration of the provocative agent compared to the baseline value, was evaluated using ANOVA for repeated measures. Comparisons of GH values or peaks among groups were evaluated using ANOVA followed by Bonferroni's test. The appropriate cut-off to differentiate case groups on the basis of the GH response to provocative tests with the highest sum of sensitivity and specificity (highest diagnostic accuracy) was established using receiver-operating characteristic (ROC) analysis. Significance was set at 5%.

Results

Baseline serum GH, IGF-I and IGF-BP3 levels were similar in the patients and controls (Table 2).

Test	Controls	MSA	PD	ILOCA
Clonidine				
Basal GH (mU/l)	1.6 ± 0.2	0.8 ± 0.1	3.4 ± 0.6	1.4 ± 0.2
GH peak (mU/l)	25.2 ± 8.4	3.4 ± 0.8*†	25.4 ± 6.6	26.6 ± 8.4
Arginine				
Basal GH (mU/l)	1.4 ± 0.2	1.2 ± 0.2	2.4 ± 0.8	2.4 ± 0.4
GH peak (mU/l)	24.4 ± 3.2	1.2 ± 0.2‡§	15.2 ± 2.2	34.6 ± 8.8
GHRH + arginine				
Basal GH (mU/l)	1.4 ± 0.1	1 ± 0.4	2.4 ± 0.6	2.4 ± 0.6
GH peak (mU/l)	51.6 ± 3.4	32.2 ± 3.6*†	45 ± 4.8	59.4 ± 7.8
Basal IGF-I (μg/l)	252.9 ± 9.9	278.8 ± 10.6	355 ± 52.1	275.9 ± 29.6
Basal IGF-BP3 (μg/l)	1.23 ± 0.01	1.22 ± 0.01	1.27 ± 0.03	1.22 ± 0.04

Table 2. Concentrations of serum GH, IGF-I and IGF-BP3 in patients with multiple system atrophy (MSA), Parkinson's disease (PD), idiopathic late-onset cerebellar ataxia (ILOCA) and controls. The values are expressed as mean ± SE

$^{*}P < 0.05$ vs. ILOCA; $^{\dagger}P < 0.01$ vs. controls; $^{\ddagger}P = 0.001$ vs. ILOCA; $^{\S}P < 0.001$ vs. controls

GH response to clonidine test

GH increased in controls, and in patients with PD and ILOCA but not in those with MSA (Table 2, Fig. 1). Moreover, the GH peak was lower in patients with MSA than in those with ILOCA ($P < 0{\cdot}05$) and in controls ($P < 0{\cdot}01$), but it was similar in patients with ILOCA and controls and in patients with MSA and PD. The standard GH peak cut-off value of 6 mU/l after clonidine test distinguished patients with MSA from those with PD with a sensitivity of 70% and a specificity of 75%. Conversely, at the optimum cut-off level of 5 mU/l, evaluated by ROC analysis, sensitivity and specificity were 80 and 75%, respectively (Fig. 2). Sensitivity and specificity were 100% and 40%, respectively, at the cut-off level of 1·6 mU/l and 40% and 100% at the cut-off of 7·6 mU/l. Moreover, at the cut-off values of 5 mU/l, the clonidine test distinguished patients with MSA from those with ILOCA with a sensitivity of 100% and a specificity of 75%, whereas at the cut-off level of 7·6 mU/l with a specificity of 100% and a sensitivity of 75% (Fig. 2).

GH response to arginine test

GH increased in controls, patients with PD and ILOCA but not in those with MSA (Table 2, Fig. 1). Moreover, GH peak was lower in patients with MSA than with ILOCA ($P < 0{\cdot}001$) and controls ($P < 0{\cdot}001$). GH peak was similar in patients with ILOCA and controls. The arginine test distinguished patients with MSA from those with PD with a sensitivity and a specificity of 75% and 100%, respectively, at the standard GH peak cut-off value of 6 mU/l whereas with a sensitivity and specificity of 100% and at the optimum cut-off value of 5 mU/l. Moreover, the test distinguished patients with MSA from those with ILOCA with sensitivity and specificity, respectively, of 75% and 100% at GH cut-off values of 6 and 5 mU/l and of 100% at a GH cut-off value of 3·6 mU/l (Fig. 2).

GH response to GHRH + arginine

GH increased in all groups (Table 2). Only one patient with MSA had an abnormal response to the test, and was considered GH deficient. When this patient was excluded from the analysis of clonidine and arginine tests, the results did not change. GH peak was significantly lower both in patients with MSA than in patients ILOCA ($P < 0{\cdot}05$) and in controls ($P < 0{\cdot}01$); it was similar in patients with ILOCA and controls (Fig. 1).

Discussion

The results of the current study demonstrated that the GH response to arginine test differentiates patients with MSA from both those with PD and with ILOCA. This suggests that this test might have a role in the differential diagnosis between MSA and both PD and ILOCA, which may display similar clinical presentation in the early stages of disease, but have a different prognosis and treatment.

MSA is a progressive neurodegenerative disease that occurs sporadically and may cause parkinsonism, cerebellar, pyramidal and autonomic dysfunction.[1] Post-mortem examination of MSA patients reveals nigrostriatal and/or olivopontocerebellar degeneration.[13] Most patients with MSA have generalized autonomic dysfunction, but this may appear only in later stages of the disease, so that an accurate early diagnosis is difficult. In particular, when MSA patients present with parkinsonism without other neurological dysfunctions, the distinction from idiopathic PD is difficult. Idiopathic PD is a common neurodegenerative disorder pathologically characterized by loss of dopaminergic neurons of the substantia nigra.[9] The differential diagnosis between MSA and PD is important, since response to antiparkinsonian treatment and prognosis in MSA are poorer. A number of diagnostic tools for distinguishing MSA from PD have been proposed but all have demonstrated a low accuracy or practicality.[4] Although parkinsonism is the most common motor disorder of patients with MSA, cerebellar features predominate in about 20% of cases.[14] When additional clinical features suggestive of MSA, such as autonomic failure and parkinsonism are unclear at initial presentation, ILOCA, a sporadic degenerative ataxia of unknown cause with a relatively pure cerebellar syndrome and a much better prognosis than MSA, can be suspected.[15] Neuropathologically, degeneration of the cerebellar cortex with loss of Purkinje cells is observed in these patients.[16] Nowadays, no clinical or instrumental test is able to accurately distinguish ILOCA from MSA in

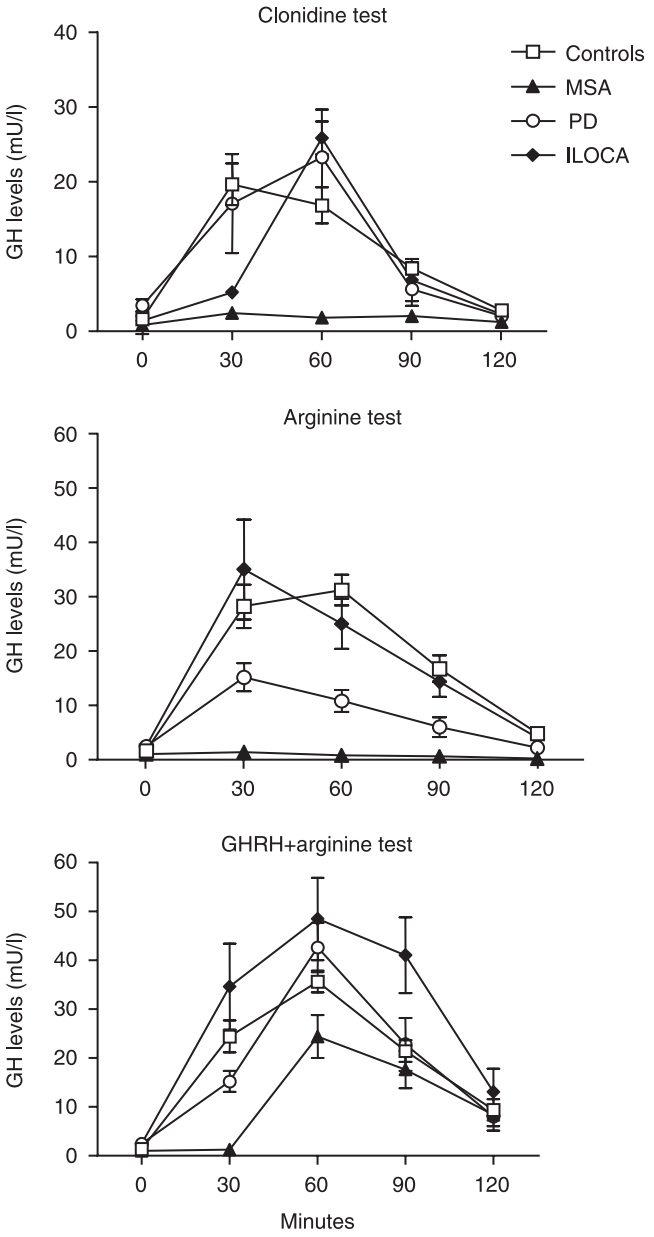

Fig. 1 Growth hormone response (mean ± SE) to clonidine, arginine and GHRH + arginine tests in controls and patients with multiple system atrophy (MSA), Parkinson's disease (PD) and idiopathic late-onset cerebellar ataxia (ILOCA).

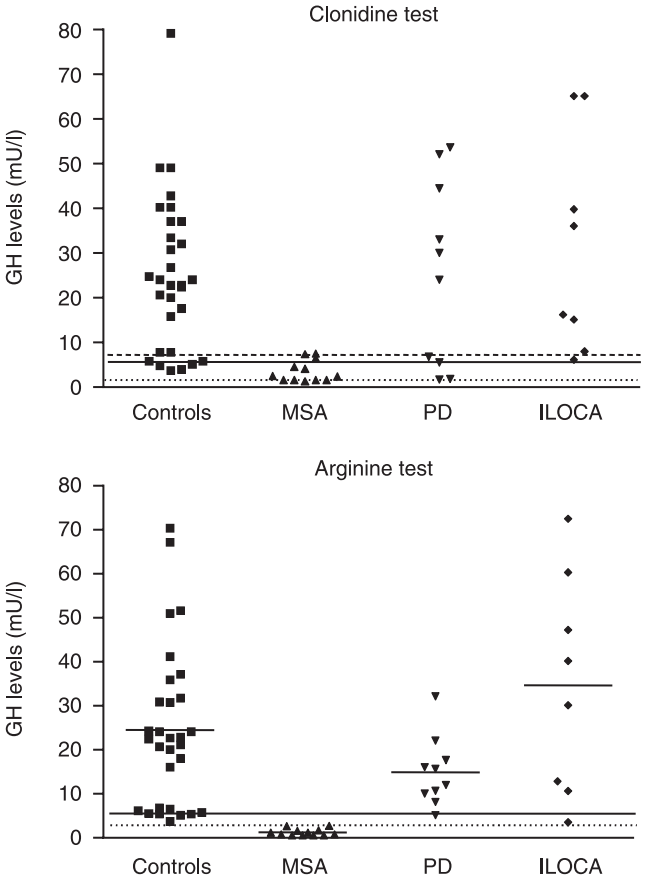

Fig. 2 Distributions of GH peaks after clonidine and arginine tests in controls and patients with multiple system atrophy (MSA), Parkinson's disease (PD) and idiopathic late-onset cerebellar ataxia (ILOCA). In both graphs the continuous line indicates the GH peak cut-off value of 5 mU/l. (*Top*) The sketched line indicates the cut-off value of 7·6 mU/l and the dotted line the cut-off value of 1·6 mU/l. (*Bottom*) The dotted line indicates the cut-off value of 3·6 mU/l.

patients who do not have specific features of the two diseases, especially in the early stages.[15]

GH secretion from the pituitary gland is regulated by a complicated neurohormonal network.[8,17] GH secretion is basically controlled through the stimulation of GH secretion by GHRH and inhibition by somatostatin. Both GHRH and somatostatin are hypothalamic neurohormones, whose pulsatile secretion is modulated by different neuronal networks, especially the noradrenergic and cholinergic systems.[8,17] In particular, the activation of hypothalamic α_2-adrenoceptors and muscarinic cholinergic receptors induces GH release, probably *via* stimulation of GHRH and inhibition of somatostatin release, respectively.[8,17] Both clonidine, an α_2-adrenoceptor agonist able to induce GH secretion through the stimulation of GHRH secretion, and arginine, an aminoacid inducing an inhibition of somatostatin release probably activating the cholinergic system, are reliable stimuli for GH secretion. Indeed, clonidine and arginine stimulation tests are two currently used tests to evaluate the pituitary GH secretion and reserve.[18] In children, a GH peak less than 20 mU/l after clonidine or arginine administration is diagnostic of GH deficiency. In adults, either clonidine or arginine are less reliable than the insulin tolerance test (ITT) and GHRH + arginine test. A GH peak less than 6 mU/l after ITT or less than 18 mU/l after GHRH+arginine test diagnoses severe GH deficiency in adults.[18] A GH peak less than 6 mU/l is considered the cut-off level for clonidine and arginine tests, although the relatively high number of false positive cases gives a low diagnostic accuracy for these two tests. However, the abolished GH response to different stimuli is not diagnostic *per se* of GH deficiency in adults, since a clinical context of hypopituitarism of known causes is required.[18] This is necessary since the GH response to pharmacological stimuli can be blunted or suppressed in different physiological or pathological conditions, such as senescence, obesity, hypothyroidism and hypercortisolism.[17] Moreover,

the circulating levels of IGF-I and IGF-BP3 may help in confirming the diagnosis of GH deficiency.[17]

The GH response to clonidine has been proposed as a good test to distinguish PD from MSA. Kimber *et al.*[4] found no rise in GH levels after clonidine in patients with MSA but a preserved response in patients with PD. Later studies did not confirm these results, and the validity of clonidine test in the differential diagnosis of these two disorders is very controversial.[5,6,19,20] Our results confirmed that there is no difference in the GH response to clonidine between MSA and PD, with an absent GH response in a great majority of patients with MSA but also in a percentage of patients with PD. This indicates that the clonidine test has a poor accuracy for the diagnosis of MSA and the differential diagnosis between MSA and PD. A loss of catecholaminergic neurons projecting to the hypothalamus could explain the lack of GH response to clonidine in patients with MSA and in a subgroup of patients with PD. This is a common finding in MSA, where a loss of catecholaminergic neurons in ventrolateral medulla has been clearly demonstrated.[21] It may also occur in PD, which combines degeneration of the dopaminergic nigral system and impairment of the catecholaminergic locus coeruleus.[6] Additionally, the results of the current study show that the GH response to clonidine has a relatively low diagnostic accuracy also in differentiating patients with MSA and those with ILOCA. Both of these diseases are characterized by a relatively pure cerebellar impairment. Therefore, the clonidine stimulation test does not likely have a significant practical diagnostic value for identifying individual patients with MSA or ILOCA.

The GH response to arginine has never been previously investigated in MSA, PD and ILOCA. In contrast to clonidine test, the arginine test showed a high sensitivity and specificity in distinguishing MSA from both PD and ILOCA, since the GH response to arginine was impaired in MSA but not in PD and ILOCA patients. The reason why the GH response to arginine is impaired in patients with MSA is unknown. However, taking into consideration that arginine induces GH secretion through the inhibition of somatostatin release and this effect is probably mediated by the cholinergic system,[22] it could be assumed that MSA, but not PD and ILOCA, is associated with damage of the cholinergic system involved in the hypothalamic control of GH secretion. Indeed, the neuroanatomical level at which cholinergic modulation affects GH release is not well defined. An intrahypothalamic cholinergic pathway that may modulate anterior pituitary function has been demonstrated in both animals and humans.[23,24] Evidence of an intrahypothalamic cholinergic defect in MSA arises from clinical and pathologic studies.[25,26] Biochemical analysis of the hypothalamus from deceased patients with MSA disclosed a marked loss of acetylcholine-transferase activity, a marker for acetylcholine containing neurons.[25] An altered vasopressin response to cholino-mimetic agents has been reported in patients with MSA, suggesting an impairment of intrahypothalamic cholinergic neurons.[26] Nevertheless, a loss of extrinsic cholinergic pathways explaining the lack of GH response to arginine in MSA cannot be ruled out. In fact, ascending projections to hypothalamus arise from brainstem cholinergic nuclei, such as pedunculopontine and laterodorsal tegmental nuclei, that are impaired in MSA.[27] Anyway, our results suggest that there is sparing of cholinergic central systems modulating GH release in PD and ILOCA, but not in MSA. By ROC analysis, the GH cut-off level after the arginine test to distinguish patients with MSA from PD with a sensitivity and a specificity of 100% was 5 mU/l, and to distinguish patients with MSA from ILOCA with similar sensitivity and specificity, was 3·6 mU/l.

It has to be emphasized that the arginine test seems, from the present data, to be more reliable in distinguishing MSA from PD and ILOCA patients than distinguishing patients with GH deficiency from normal subjects, according to other data in the literature. This may be due to the different GH cut-off used for the two different purposes, to the extremely low postarginine GH values constantly documented in patients with MSA and to the relatively high postarginine GH values constantly documented in patients with PD. In any event, the exact role of the arginine test and the optimum cut-off of GH values that can eventually be used in the differential diagnosis of MSA and PD needs further research in a larger number of patients. The reason for the peculiar response of GH to arginine in these two neurological diseases also needs further investigation.

Finally, the GHRH + arginine test appears to have no role since this test induces a maximal response mediated by an intact pituitary gland in all subjects studied, and cannot be used for the purpose of differential diagnosis among MSA, PD and ILOCA. However, the normal GH response to this latter test in all patients except one and the normal levels of IGF-I and IGFBP-3 in all patients of the study, allows exclusion of GH deficiency in our subjects. Exclusion of the one subject who failed the GHRH-arginine test did not change the results and the meaning of our study.

In conclusion, the current study demonstrated that the GH response to the arginine test has a higher sensitivity and specificity than GH response to clonidine in differentiating patients with MSA from those with PD and ILOCA. The preliminary results of the current study suggest that at the GH cut-off levels of 5 and 3·6 mU/l, respectively, the arginine test could be a simple and clinically useful tool for the differential diagnosis of individual patients with MSA from those with PD and ILOCA. However, further studies on a large population of patients with these neurological diseases are mandatory to confirm these findings.

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