Short report

Serum insulin-like growth factor-I levels in amyotrophic lateral sclerosis

GLENN D BRAUNSTEIN, ANTIA L REVICZKY

From the Department of Medicine, Cedars-Sinai Medical Center-UCLA School of Medicine, Los Angeles, USA

SUMMARY The serum concentrations of the myotrophic hormone insulin-like growth factor-I (IGF-I) in 23 patients with amyotrophic lateral sclerosis were not significantly different from those found in the sera of 13 control patients. There was no difference in binding of ^{125}I -IGF-I by serum from patients with amyotrophic lateral sclerosis in comparison with that found in the controls. These results indicate that immunoreactive IGF-I concentrations are normal in patients with amyotrophic lateral sclerosis and that such patients do not have significant antibodies binding their endogenous IGF-I.

Several tissue growth factors have been shown to stimulate amino acid uptake and protein synthesis in muscle cells.¹⁻³ The muscle atrophy found in some neuromuscular diseases could be due to deficient secretion or action of one or more of these tissue growth factors. One such growth factor, insulin-like growth factor-I (IGF-I, somatomedin-C) has attracted interest because it is the primary growth-promoting mediator of growth hormone action and low levels have been found in some disease states associated with muscle weakness.⁴ Therefore, we examined the serum concentrations of immunoreactive IGF-I in patients with amyotrophic lateral sclerosis.

Materials and methods

Patients

Fasting blood samples were obtained from 23 patients with amyotrophic lateral sclerosis and 13 control subjects of similar age. The diagnosis of amyotrophic lateral sclerosis was made by at least one neurologist in all cases, and was based on findings of diffuse muscle weakness, atrophy and fasciculations associated with corticospinal and/or corticobulbar tract signs without involvement of sensation, coordination, or cognitive function. The diagnosis was supported by electromyography and histochemical studies

of representative skeletal muscles in the majority of patients. All of the amyotrophic lateral sclerosis patients were well-nourished, although 12/23 were non-ambulatory (table). Blood was obtained between 08:00 and 09:00 h following an overnight fast. The serum was rapidly separated from the clot and stored at -20°C until assay.

IGF-I measurements

IGF-I was measured by two methods with reagents generously supplied by the National Hormone and Pituitary Program (Baltimore, Maryland). The first assay utilised the borosilicate-protamine-free assay system of Furlanetto *et al*⁵ to measure IGF-I in unextracted serum. The second assay used polystyrene tubes containing protamine⁶ to measure IGF-I in acid-ethanol extracted serum samples. As antigen for the radiolabelling, and as standard, a recombinant human IGF-I (Thr 59) analog (Amgen, Thousand Oaks, CA) was used. This analog has been shown to displace ^{125}I -IGF-I binding in parallel with human serum-derived somatomedin-C and IGF-I synthesised by solid phase.⁷

Recovery of added IGF-I in these assays averaged $93.4\% \pm 14.7\text{ SD}$ ($n = 9$). The within assay coefficients of variation varied between 11 and 12.6% while the between assay coefficients of variation ranged from 14 to 17.7%. All samples were run in duplicate in the same assay, and each assay contained control samples with both high IGF-I (acromegalic serum) and low IGF-I (hypopituitary serum) levels.

Examination of serum samples for IGF-I antibodies

Two methods were employed to examine serum samples for the presence of antibodies against IGF-I. In the first, 200 μl of patient's serum, 100 μl of ^{125}I -IGF-I (10,000 cpm) and 200 μl of phosphate buffer saline, pH 7.8 containing 1% normal rabbit serum were mixed and incubated overnight at room temperature. Antigen-antibody complexes were pre-

Address for reprint requests: Glenn D Braunstein, MD, Room 2119 North Tower, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Los Angeles, CA 90048, USA.

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Table IGF-I levels and anti-IGF-I activity in patients with amyotrophic lateral sclerosis

Patient	Sex	Age (yr)	Duration of ALS (yr)	Ambulatory	IGF-I		Anti-IGF-I activity (% bound)
					Borosilicate protamine free (units/ml)	Polystyrene-protamine (units/ml)	
1	M	27	3.5	Yes	0.74	1.01	2.66
2	F	54	0.25	Yes	0.49	0.54	0.22
3	M	42	2	No	0.21	0.42	3.52
4	M	84	2.75	Yes	1.10	0.92	2.6
5	M	62	8	No	0.67	0.81	1.21
6	F	67	6	No	0.69	0.57	0.85
7	M	72	14	No	1.24	0.91	2.41
8	M	62	1	Yes	0.63	0.78	1.82
9	M	32	2.5	Yes	0.66	0.85	1.81
10	F	50	0.5	No	0.60	0.58	1.61
11	M	70	2	No	1.12	0.88	0.74
12	M	62	2	No	0.84	0.86	1.72
13	F	58	5	Yes	0.94	0.95	1.56
14	M	55	2	Yes	1.66	1.27	1.41
15	M	39	1.25	No	1.38	1.02	1.51
16	M	66	4.5	Yes	0.58	0.64	2.45
17	F	41	7.5	No	1.01	0.84	1.10
18	F	66	6	No	0.89	1.07	1.51
19	M	67	3	No	1.13	0.82	—
20	M	71	5	Yes	0.86	0.86	—
21	M	40	3	Yes	1.02	0.92	—
22	M	60	2	Yes	0.99	1.3	—
23	M	72	10	No	0.68	0.69	—
Mean $\pm$ SD		57.3 $\pm$ 14.6	4.1 $\pm$ 3.3		0.88 $\pm$ 0.32	0.84 $\pm$ 0.2	1.71 $\pm$ 0.8
Controls							
mean $\pm$ SD		56.2 $\pm$ 13.9			0.87 $\pm$ 0.52	0.88 $\pm$ 0.36	2.31 $\pm$ 0.9
(Range)		(27–77)			(0.4–2.05)	(0.45–1.51)	(1.41–3.29)

precipitated by the addition of 1 ml 15% polyethylene glycol (molecular weight 6,000). After centrifugation, the supernatant was aspirated and the radioactivity present in the precipitates was counted. Nonspecific binding was assessed by the addition of excess unlabelled IGF-I (100 ng) to the assay tubes prior to incubation. The second method employed the same incubation conditions, but differed from the first in that antigen-antibody complexes were precipitated by rabbit anti-human IgG antibody by the addition of Immuno-beads (400 mcg anti-IgG per tube) (Bio-Rad Laboratories, Richmond, CA 94804).

Statistics

IGF-I levels in the patient and control sera were compared by *t* test and significant differences were considered to be present when $p < 0.05$. Correlations between IGF-I levels measured in unextracted and extracted serum was carried out by least square linear regression analysis.

Results

The individual and mean serum IGF-I values measured in unextracted and extracted samples in the amyotrophic lateral sclerosis patients and controls are presented in the table. There were no significant differences in IGF-I levels measured by either method between the patients and controls nor was there a sex difference in levels within or between the groups. There were no significant differences between IGF-I concentrations measured by the borosilicate tube-protamine free buffer assay or the polystyrene-tube

protamine buffer method. The correlation of IGF-I values by the two methods in each of the serum samples was highly significant ($r = 0.88$, $p < 0.001$).

Most of the serum samples had less than 3% specific binding of ^{125}I -IGF-I (table). There were no differences between the serum anti-IGF-I binding activity detected by either the polyethylene glycol method or by the Immuno-bead method (data not shown) between patients with amyotrophic lateral sclerosis and control patients.

Discussion

The aetiology of amyotrophic lateral sclerosis and the pathogenesis of its abnormalities are unclear. Genetic, toxic, nutritional, hormonal, viral, and immunologic factors have all been implicated in the pathogenesis and progression of the disease.⁸ Gurney and co-workers described an antibody in the sera of patients with amyotrophic lateral sclerosis that inhibits sprouting of neurons and reinnervation of skeletal muscle.⁹ This observation has raised the possibility of abnormalities in the action of neuromuscular trophic factors in amyotrophic lateral sclerosis. It is therefore conceivable that abnormalities of other tissue trophic factors may be of importance in the development of the muscular atrophy seen in amyotrophic lateral sclerosis.

The present study demonstrates that serum levels

of IGF-I in patients with amyotrophic lateral sclerosis are not different from a control population. In addition, we were unable to demonstrate the presence of serum antibodies against IGF-I. These results, however, do not negate the possibility that the biological activity of IGF-I may be abnormal in patients with amyotrophic lateral sclerosis. Impaired biological activity may be due to the presence of circulating inhibitors of IGF-I action, which are known to be present in other chronic diseases.¹⁰ Alternatively, IGF-I binding to its specific muscle receptor¹¹ may be altered, or a postreceptor defect may result in altered IGF-I activity at the level of the muscle and/or nerve cell.

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