SUBCLINICAL AUTONOMIC DYSFUNCTION IN SPINOBULBAR MUSCULAR ATROPHY (KENNEDY DISEASE)

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ABSTRACT: *Introduction:* Spinobulbar muscular atrophy (SBMA) is an inherited adult-onset motor neuron disease caused by the expansion of a polyglutamine tract within the androgen receptor. Autonomic nervous system involvement (ANS) is not considered part of SBMA. The aim of this study was to assess autonomic cardiovascular function in 5 SBMA patients. *Methods:* Five quantitative autonomic function tests (AFTs) were performed in 5 SBMA patients. Plasma noradrenaline (NA) concentration in patients and in 5 healthy subjects was also measured. *Results:* AFTs were abnormal in 4 of the 5 patients, and plasma NA concentration was significantly reduced in patients with respect to controls. *Conclusion:* The impairment of cardiovascular responses to AFTs in addition to reduced plasma NA concentration observed in our patients suggests subclinical involvement of the ANS in Kennedy disease.

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Spinobulbar muscular atrophy (SBMA) is an inherited adult-onset motor neuron disease caused by expansion of a polyglutamine (polyQ) tract within the androgen receptor and affects only males.[1] Kennedy disease is presently considered a sensorimotor neuronopathy,[2] although it is conventionally reported as an adult-onset motor neuron disease.[3] SBMA patients develop premature muscular exhaustion and subsequently have slowly progressive muscle weakness and fasciculation in bulbar and limb muscles, whereas sensory disturbances are often unremarkable.[4] Autonomic nervous system (ANS) involvement is not considered to be part of SBMA. However, autonomic skin denervation has been reported in 2 patients with SBMA.[5] In this study we investigated autonomic cardiovascular function using autonomic function tests (AFTs) and measurements of plasma noradrenaline concentration.

METHODS

Five patients with genetically confirmed SBMA, aged 39–55 years, participated in the study (Table 1). Patients with diabetes, glucose impairment, heart disease, or hypertension were excluded. None of them had clinical evidence of dysautonomia. Subjects were studied in a temperature-controlled room (23 ± 1°C). None of the patients had been taking medication known to affect autonomic function, and they were asked to abstain from alcohol and caffeine for at least 24 hours before the investigations. Continuous non-invasive measurement of systolic and diastolic blood pressure (SBP, DBP) was obtained by an infrared photoplethysmograph (Model 1 Finometer; TNO Biomedical Instrumentation, Amsterdam, The Netherlands). Electrocardiographic assessment (Click ECG USB 3–12 leads; ET Medical Devices SpA, Cavareno, Italy) was performed using standard methods. Respiration was also monitored continuously using a nasal thermocouple respiration flow sensor (SleepSense). A cannula was inserted into a vein in the antecubital fossa for blood sampling to evaluate plasma noradrenaline (NA) concentration. AFTs were performed using standard procedures.[6] The tests were performed in the order outlined in what follows, allowing for a period of rest to reach basal blood pressure (BP) and heart rate (HR) values in between investigations. The results of each test were automatically obtained using Light-SNV software. Five healthy, male volunteers, aged 33–54 years, served as controls for plasma NA measurements. All subjects provided informed consent to the procedures, and the study was approved by the local ethics committee.

Head-Up Tilt Test. After 30 minutes of supine rest, each subject was tilted up at 65° on a tilt table for 10 minutes. At each minute of the head-up tilt test (HUTT), changes in SBP, DBP, and HR were calculated with respect to basal values. Pre-HUTT supine values (baseline) for SBP, DBP, and HR were set at 0, and changes expressed as change (raw data) from baseline. Abnormal responses were defined as a decrease in SBP of ≥20 mm Hg or in DBP of ≥10 mm Hg or an increment of HR ≥30 beats per minute (bpm).

Valsalva Maneuver. The Valsalva maneuver was performed by blowing through a mouthpiece attached to a manometer and maintaining a pressure of

Abbreviations: AFT, autonomic function test; ALS, amyotrophic lateral sclerosis; ANS, autonomic nervous system; BP, blood pressure; DBP, diastolic blood pressure; EGTA, ethylene-glycol tetraacetic acid; HPLC, high-pressure liquid chromatography; HR, heart rate; HUTT head-up tilt test; IE difference, inspiration–expiration difference; NA, noradrenaline; OV, overshoot; polyQ, polyglutamine; SBMA, spinobulbar muscular atrophy; SBP, systolic blood pressure; VR, Valsalva ratio

Key words: androgen receptors, autonomic dysfunction, autonomic function tests, Kennedy disease, plasma noradrenaline

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Table 1. Clinical, genetic, and electrophysiological data.

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5
Age at examination (y)	55	48	48	39	32
Age at onset (y)	50	43	45	35	28
CAG repeat number	43	46	43	49	48
Muscle weakness					
Bulbar	++	++	+	++	+
Upper limb	+	+	+	+	+
Lower limb	+	++	+	++	+
Fasciculations	++	++	+	++	++
Sensory signs	None	None	None	None	None
Gynecomastia	A	A	A	+	A
CK (U/L)	1500	560	622	4396	624
CMAP amplitude (mV)					
Ulnar	6.9	10.1	10.6	5.8	7.6
Median	5.1	7.9	8.2	3.7	7.1
Fibular	3.2	8.4	7.9	3.8	5.0
SNAP amplitude (μV)					
Median	2.6	4.5	3.5	5.3	5.1
Ulnar	A	1.9	3.1	3.3	4.9
Sural	1.6	2.2	4.3	A	3.7

Normal values for serum creatine kinase (CK): 10–167 U/L. Normal limits for compound motor action potentials (CMAPs): 4 mV for fibular nerve; 6 mV for ulnar nerve; and 4 mV for median nerve. Normal limits for sensory nerve action potentials (SNAPs): 20 μV for median nerve; 18 μV for ulnar nerve; and 6 μV for sural nerve. Pt, patient; +, mild; ++, moderate; A, absent.

40 mm Hg for 15 seconds. The maneuver has four phases.[7] Phases I and III are purely mechanical, reflecting intrathoracic pressure changes. We considered, as an index of autonomic activity, the Valsalva ratio (VR), which is the ratio between HR in phases II and IV and the BP variations during phases II and IV. We considered a VR >1.21 to be a normal value. There are no normative BP values for phase II and phase IV overshoot. There is great variability between subjects, but we considered an abnormal result to be loss of recovery of SBP in late phase II and absence of overshoot (OV).

Deep Breathing Test. Each subject breathed deeply 6 times per minute while supine. A total of 10 respiratory cycles were recorded. Sinus arrhythmia was calculated in beats per minute (bpm). The difference between the maximum HR during inspiration and minimum HR during expiration (IE difference) in an individual respiratory cycle was calculated and expressed as the mean of differences in 10 respiratory cycles. The normal value for IE difference is $\geq$15 bpm.

Handgrip Test. Each patient was asked to exert 30% maximal voluntary contraction of the dominant hand for 3 minutes on a dynamometer. BP was measured in the non-exercising arm at rest and at the third minute of the test. A rise in DBP of >15 mm Hg was considered to be normal.

Plasma Noradrenaline Measurement. A 16G Teflon catheter was inserted 30 minutes before testing, and 5-ml blood samples were taken after 10 and 15 minutes supine and 10 minutes tilting for measurement of plasma NA concentration. Samples were immediately mixed with 0.1 ml of ethylene-glycol tetraacetic acid (EGTA)/reduced glutathione and stored on ice before centrifugation (3000 rpm for 10 minutes at 4°C). Plasma was immediately pipetted off, frozen, and stored before measurement of NA concentrations with high-pressure liquid chromatography (HPLC) with electrochemical detection (ICS 3000; Dionex).[8] Comparison between patients and control groups was evaluated by Welch's approximate test for population with different standard deviations. $P < 0.05$ was considered significant.

RESULTS

Autonomic Function Tests. The sympathetic nervous system was assessed by the blood pressure responses to HUTT, to Valsalva maneuvers, and to the handgrip test. The parasympathetic cardiovagal axis was assessed by heart rate variation during deep breathing and by the Valsalva ratio. All subjects correctly performed all the autonomic tests. The basal values of SBP, DBP, and HR during supine rest were normal. During HUTT different SBP and DBP responses were observed. Two of 5 patients (patients 1 and 5) showed a decrease in SBP of >20 mm Hg or a decrease in DBP of >10 mm Hg associated with an insignificant increase in heart rate (Table 2). The same patients had an abnormal increase in DBP in the handgrip test and had an absence of late phase II and phase IV in the Valsalva maneuver. The cardiovascular responses to deep breathing were abnormal in patients 2 and 3. Patient 3 also had a pathologic

Table 2. Autonomic function test results.

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5
Supine rest					
SBP (mm Hg)	130	110	134	128	127
DBP (mm Hg)	80	70	83	78	69
HR (bpm)	74	60	61	67	71
Differences after 10-min tilt					
SBP (mm Hg)	−10	28	−4	−17	−30*
DBP (mm Hg)	−16*	16	2	−7	−15*
HR (bpm)	5	17	16	20	3
Valsalva					
II L (mm Hg)	A*	2	1	3	A*
OV (mm Hg)	A*	51	9	30	A*
VR	1.49	1.46	1.35	2.03	1.21
Deep breathing					
IE difference (bpm)	19	11*	11*	16	38
Handgrip					
ΔDBP (mm Hg)	9*	26	9*	15	8*

Pt, patient; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; II L, SBP variation in phase II late; OV, overshoot on phase IV; VR, Valsalva ratio; IE, inspiration–expiration difference; ΔDBP, changes in diastolic blood pressure; A, absent.
**Pathologic values.*

Table 3. Plasma noradrenaline concentration (pg/ml).

	Lying NA*	ΔNA†
Patient 1	646.6	−116.6
Patient 2	135	105
Patient 3	511.2	16.8
Patient 4	247.8	119.7
Patient 5	222.8	5.2
Patients (mean ± SD)	352.7 ± 216.1	26 ± 94.7
Controls (mean ± SD)	778 ± 260.5	561.5 ± 428.5
P‡	<0.03	<0.05

*Noradrenaline concentration at rest (mean of two values obtained at 5 and 15 minutes of supine rest).
†Noradrenaline variation after 10 minutes of head-up tilting.
‡Welch's test.

response to the handgrip test. Patient 4 had normal responses to all AFTs.

Plasma Noradrenaline. The plasma NA increase in response to HUTT was significantly reduced ($P < 0.05$) in the patient group compared with the control group. A significant difference in lying ($P < 0.03$) plasma NA concentration between patients and controls was also detected (Table 3).

DISCUSSION

Our results indicate that the cardiovascular responses to certain physiological stimuli were impaired in patients with Kennedy disease. Two of 5 patients had asymptomatic postural hypotension[9] on HUTT. One reason for postural hypotension is sympathetic vasoconstrictor failure, as it occurs in patients with primary autonomic failure, with central or peripheral denervation.[6] Furthermore, the reduced DBP response to the handgrip test and the absence of phase II late and phase IV in the Valsalva maneuver represent additional signs of adrenergic impairment.[10] In fact, phase IV seems to be more dependent on cardiac adrenergic tone, and phase II late is an important determinant of α-adrenergic function as the most reliable index of systemic peripheral resistence.[11] Two of 5 patients showed a decreased heart rate response to deep breathing test but had a normal VR. This would be in favor of a vagal efferent abnormality.[10]

The lack of an NA rise with tilt in the patient group suggests baroreflex–sympathoneural failure similar to what occurs in multiple system atrophy, Parkinson disease with orthostatic hypotension, and pure autonomic failure.[12] The low basal plasma levels of NA during supine rest may suggest lack of NA release at sympathetic endings due to peripheral autonomic failure, as demonstrated in pure autonomic failure and autoimmune autonomic ganglionopathy.[12,13] The fact that NA concentration was reduced in the patient group while AFT assessments of the sympathetic nervous system were not abnormal in all patients could depend on the low sensitivity of physiological tests for detection of subclinical abnormalities. ANS involvement is not considered part of motor neuron diseases, although subtle ANS alterations have been described in amyotrophic lateral sclerosis (ALS).[14,15] A case of autonomic failure due to lesions of the autonomic nuclei was reported in ALS with a novel SOD1 gene mutation.[16] Our data could be explained by the presence of diffuse nuclear accumulations of mutant androgen receptor in the intermediolateral nucleus of the spinal cord and sympathetic ganglia and in the nucleus ambiguus where vagal cardiomotor neurons are located.[17] Moreover, widespread nuclear accumulations of mutant androgen receptor were found in various regions of the central autonomic network, including hypothalamus and periaqueductal gray.[17] These are regions that are implicated in the regulation of arterial pressure, sympathetic activity, and heart rate.[18,19] Abnormality of autonomic function tests and reduction of NA concentration did not correlate with duration, severity, clinical course of disease, CAG repeat size, or age of patients. Our findings reinforce the view that there is subclinical involvement of the ANS, including cardiovascular autonomic function, in Kennedy disease, and thus SBMA is a multisystem disease. Nevertheless, additional and more extensive studies are required.

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