Botulism: Heart Rate Variation, Sympathetic Skin Responses, and Plasma Norepinephrine

J.T. Chen, C.C. Chen, K.P. Lin, S.J. Wang, Z.A. Wu and K.K. Liao

ABSTRACT: ***Background:*** Botulism may involve the autonomic nervous system. ***Methods:*** We assessed the autonomic function of 6 botulism patients with heart rate variations, sympathetic skin responses, and plasma norepinephrine. ***Results:*** Two weeks after onset, all the patients had absent sympathetic skin response in the palm and sole. Compared with controls, the heart rate variation of botulism patients was significantly decreased at rest (3.1 ± 1.2% vs. 20.9 ± 2.0%, $p = 0.0018$) and during deep breathing (4.3 ± 2.3% vs. 29.7 ± 2.6%, $p = 0.0018$). The botulism patients had significantly lower plasma norepinephrine levels (supine 29.2 ± 10.1 pg/ml vs. 257.5 ± 65.8 pg/ml, $p = 0.0018$; standing 40.3 ± 13.1 pg/ml vs. 498.5 ± 85.6 pg/ml, $p = 0.0018$). The heart rate variation and sympathetic skin response was greatly improved 6 months after onset. ***Conclusions:*** Heart rate variation, absence of sympathetic skin response, and low plasma norepinephrine are all manifestations of autonomic dysfunction in botulism patients.

RÉSUMÉ: Le botulisme: variation du rythme cardiaque, réponses cutanées sympathiques et norépinéphrine plasmatique. ***Introduction:*** Le botulisme peut toucher le système nerveux autonome. ***Méthodes:*** Nous avons mesuré les variations du rythme cardiaque, les réponses cutanées sympathiques et la norépinéphrine plasmatique pour évaluer la fonction du système nerveux autonome chez 6 patients atteints de botulisme. ***Résultats:*** Deux semaines après le début de la maladie, tous les patients présentaient une absence de réponse cutanée sympathique au niveau de la paume des mains et de la plante des pieds. La variation du rythme cardiaque des patients était significativement diminuée au repos par rapport aux contrôles (3.1 1.2% vs 20.9 2.0%, p = 0.0018) et pendant la respiration profonde (4.3 2.3% vs 29.7 2.6%, p = 0.0018). Les patients atteints de botulisme avaient des niveaux significativement plus bas de norépinéphrine plasmatique (29.2 ± 10.1 pg/ml vs 257.5± 65.8 pg/ml en décubitus dorsal, p = 0.0018; 40.3 ± 13.1 pg/ml vs 498.5 ± 85.6 pg/ml en position debout, p = 0.0018). La variation du rythme cardiaque et la réponse cutanée sympathique se sont beaucoup améliorées 6 mois après le début de la maladie. ***Conclusions:*** Notre étude suggère que la variation du rythme cardiaque, la réponse cutanée sympathique et la norépinéphrine plasmatique pourraient refléter la dysfonction neurovégétative chez les patients atteints de botulisme.

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Botulinum toxin impairs the release of acetylcholine from nerve terminals. It may act on ganglionic nerve endings, postganglionic parasympathetic nerve endings, postganglionic sympathetic nerve endings,[1-3] and some adrenergic and nonadrenergic atropine resistant autonomic neuromuscular sites.[4] The postganglionic noradrenergic function is preponderantly affected by botulism B[5] and can be reflected by the plasma norepinephrine response.[6]

R-R interval variation (RRIV) and sympathetic skin response (SSR) have been used to assess autonomic function.[7-9] These tests are easy to perform in an EMG laboratory and are good adjuncts to the assessment of autonomic function. RRIV is mainly indicative of the parasympathetic function of the vagus nerve,[7] whereas SSR represents the function of sympathetic sudomotor fibers.[10-11] In this study, we evaluated the autonomic functions of botulism patients with RRIV, SSR, and plasma norepinephrine.

Materials and Methods

Subjects

After informed consent, six botulism B patients (1 male, 5 female; age ranging from 42 to 56 years, mean age 49.3 years) and 9 age-matched normal subjects were enrolled in the study. The botulism patients were all from the same outbreak. Botulism B toxin was identified in the stool and serum. They presented with botulism after ingestion of contaminated canned food. The latent period varied from 12 to 22 hours. Their clinical manifestations

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Table: Autonomic function tests and electrophysiological findings of botulism patients 2 weeks after onset.

Case	Sex/Age	Distal latency# (3.5 ± 0.4ms)	CMAP# (11.9 ± 3.7mV)	3Hz RT# (< 10%)	30Hz RT# (< 50%)	rest/DB RRIV(%) (20.9 ± 2.0/29.7 ± 2.6)	SSR (palm/sole) (present/present)	supine/standing NE (257.5 ± 65.8/498.5 ± 85.6pg/ml)	Orthrostatic hypotension
1	F/51	5.0*	1.0*	–9%	+25%	1.5*/1.9*	absent/absent	48*/65*	present
2	F/44	2.7	1.5*	–8.8%	+25%	3.1*/3.1*	absent/absent	25*/33*	present
3	M/51	4.1	2.5*	0%	+133%**	4.4*/8.5*	absent/absent	17*/22*	present
4	F/52	4.8*	4.0*	–7.7%	+51.3%**	4.6*/7.7*	absent/absent	26*/40*	present
5	F/56	5.4*	8.5	–5%	+20%	1.5*/1.9*	absent/absent	28*/38*	present
6	F/42	3.7	8.8	–3%	+41%	4.6*/4.7*	absent/absent	30*/42*	present

#: data of median nerve with stimulation at wrist and recording at abductor pollicis brevis; CMAP: compound motor action potentials; *: two standard deviations above or beyond the mean value of control; **: incremental response more than 50%; –: decrement in CMAP amplitude; +: increment in CMAP amplitude; RT: repetitive test; DB: deep breathing; RRIV: R-R interval variation; SSR: sympathetic skin response; NE: norepinephrine.

were dysphonia, ptosis, blurred vision, dry eyes and mouth, palpitation, persistent dizziness, postural hypotension, dyspnoea, generalized weakness, anhidrosis, urinary retention, nausea, vomiting and abdominal fullness. Their pupils were dilated and failed to react to light. None of the patients had a history of diabetes, heart disease, alcoholism, or other disorders that might affect the autonomic nervous system. During our study, none of the patients received any medications that could act on the autonomic or cardiovascular system. We also performed nerve conduction studies, needle electromyography and repetitive tests to assess the degree of paralysis of the patients. Electrophysiological studies showed: (1) a mild, prolonged distal latencies or low amplitude of compound motor action potentials in 5 of 6 patients; (2) incremental response at 30 Hz of stimulation in 2 of 6 patients. The repetitive test and motor nerve conduction study of median nerve (Table) was done with stimulation at wrist and recording at abductor pollicis brevis. Before the study, all patients had spirometric tests to make sure that their pulmonary functions had recovered. Our studies were usually done on the 14th and 15th days after onset. At that time, all patients could arise and stand with support.

During the study of SSR and RRIV, all subjects were asked to relax and to lie on a couch in a dimly lit and quiet room. The room temperature was kept between 22°C and 24°C. Skin temperature was kept above 31°C. The skin surface was carefully cleaned prior to placing the surface electrodes, which were tightly apposed to the skin with electrolyte gels.

SSR

Surface electrodes were placed on the palm (G1) and dorsum (G2) of the right hand and on the sole (G1) and dorsum (G2) of the right foot. A filter setting was used, including a 0.5-1000 Hz bandpass, a sensitivity of 0.5 to 3 mV per division, and a sweep speed of 1.0 second per division. Stimuli delivered at the left wrist consisted of single square pulses of 0.2 ms duration and 200 V intensity. The stimuli were given at irregular intervals. Amplitude and latency of individual responses were not assessed as they varied greatly with consecutive stimuli. The response was considered absent if no consistent voltage change was detected using a sensitivity of 50 μV per division after 6 or more trials to avoid habituation of the response.

Heart rate variation

According to our previously published methods,[8,9] RRIV measurements were recorded during rest and deep breathing (DB). Surface recordings were made using two disk electrodes placed on the chest wall across the cardiac position with a ground electrode on the right wrist. After using the triggering mode and adjusting the sweep speed, two QRS complexes were simultaneously displayed on the screen. The first displayed complex was the triggering potential, and the variation in timing of the second complex represented the variation in timing of the R-R interval. Twenty sweeps were recorded, superimposed and measured on the screen. Five groups of 20 sweeps were recorded as rest RRIV after 5-minutes rest in supine position. Two groups were recorded as DB RRIV, with forced deep breathing at 6 breaths per minute, allowing 5 seconds for inspiration and 5 seconds for expiration. The mean R-R interval (a) and the range of R-R interval (b) was calculated as the difference between them. RRIV was defined as a percentage of the average R-R interval using the formula: RRIV = b/a x 100%.

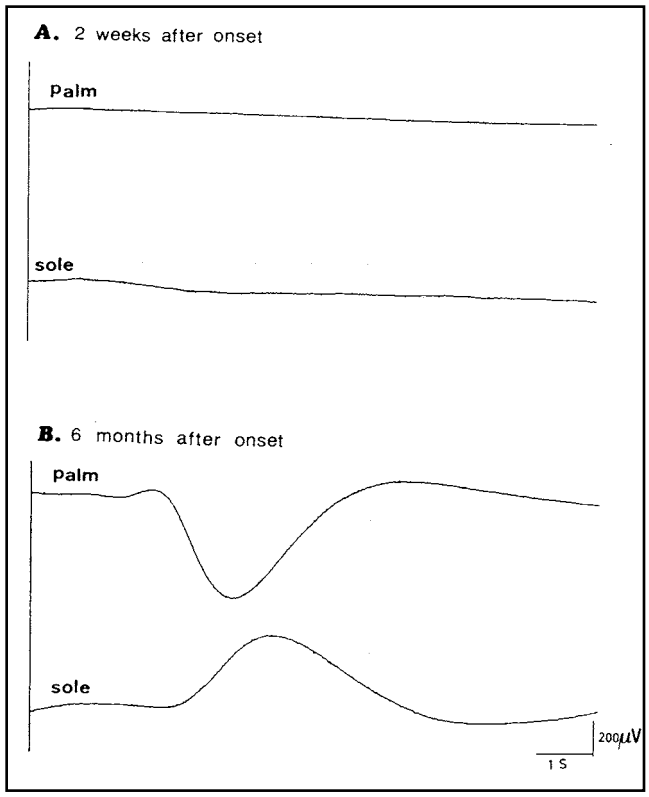

Figure 1: *SSR of a botulism patient. A. Two weeks after onset, the SSR was absent in the palm and sole. B. Six months after onset.*

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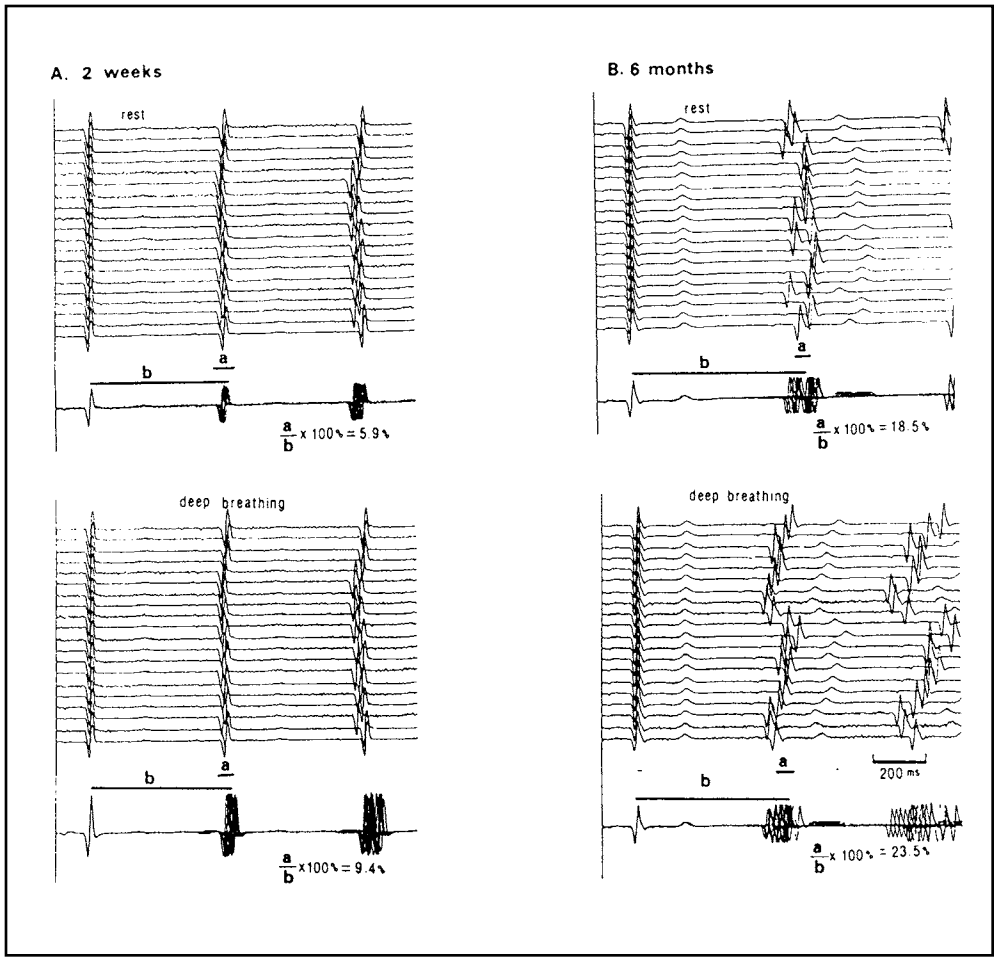

Figure 2: *A. Two weeks after onset, the RRIV of a botulism patient ranged from 3.6 to 5.9% at rest and 6.6% to 9.4% during deep breathing. B. Six months after onset, the RRIV ranged from 13.6% to 19.4% at rest and 21.1% to 26.5% during deep breathing.*

Plasma norepinephrine

A forearm vein was cannulated for collection of blood for the measurement of plasma norepinephrine. Subjects rested recumbent for at least 20 minutes after venopuncture with a heparin lock before the first blood sample was obtained. The second sample was taken after they had stood erect for 5 minutes. All patients were helped to rise up and were supported in the upright posture during the test. They were allowed to sit down if they could no longer tolerate the upright posture. Blood samples were collected in heparinized tubes and centrifuged immediately, and the plasma was stored at 20°C until assayed. Plasma norepinephrine was measured by high performance liquid chromatography with an electrochemical detector.

Statistical analysis

Analysis of variance was used to compare results of plasma norepinephrine levels in supine and standing patients, and to compare the heart rate variation at rest and during deep breathing. A comparison between patient group and normal subjects was made using the Wilcoxon Rank Sums test. Probability (p) value < 0.05 was considered statistically significant.

Results

SSR

The results of SSR and RRIV are shown in the Table. SSR abnormalities were defined as an absence of response in either the palm or sole, because every control subject for SSR displayed a response in both the hand and foot. Our patients had absent SSR in both the hand and foot two weeks after onset and had normal studies of SSR 6 months later (Figure 1).

Heart rate variation

Compared with controls (at rest, range 15.6 - 24.6%, mean 20.9 ± 2.0%; DB, range 26.8 - 33.8%, mean 29.7 ± 2.6%), the RRIV of botulism patients was significantly decreased at rest (range 1.5 - 4.6%, mean 3.1 ± 1.2%, $p = 0.0018$) and during DB (range 1.9 - 7.7%, mean 4.3 ± 2.3%, $p = 0.0018$) two weeks after onset (Table) and improved 6 months later (Figure 2).

Norepinephrine

The botulism patients had a significantly lower plasma norepinephrine level (supine 29.2 ± 10.1 pg/ml vs. 257.5 ± 65.8 pg/ml; $p = 0.0018$; standing 40.3 ± 13.1 pg/ml vs. 498.5 ± 85.6 pg/ml; $p = 0.0018$).

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Discussion

Botulism is characterized by muscle weakness, acute autonomic dysfunction, and gastrointestinal symptoms. It is caused by toxins produced by the organism *Clostridium botulinum*.[5] Intoxication may occur in three forms; food-borne, wound, and infant botulism. All our patients were of the food-borne type. Autonomic dysfunction of botulism is characterized by widespread involvement of parasympathetic and sympathetic, ganglionic and postganglionic,[1-3] adrenergic and nonadrenergic neuromuscular sites.[4]

Reduced heart rate variability was noted in botulism patients at rest and during deep respiration. Heart rate variability is easily provoked by deep inspiration. This variability is mediated via the baroreflex through cardiovagal control. Thus, reduction of the effect of respiration on heart rate indicates cardiovagal dysfunction. It also correlates well with its severity.[12] In the cardiovagal reflex, there are sympathetic and parasympathetic components. In the botulism study of Vita et al.,[13] the sympathetic function became normal earlier than parasympathetic function. It seemed that the primary effect of botulism was on the parasympathetic cholinergic synapses. Abnormal cardiovagal function is a predisposing factor of arrhythmia. It may account for cardiac arrhythmia and sudden death of some botulism patients.[14]

Absent SSR was noted in botulism patients. SSR can be evoked by electrical stimulation of peripheral nerve and can occur spontaneously.[15] SSR was thought to be the result of a synchronized activity of sweat glands. Thus, SSR is used to be an index of sudomotor function. The absence of SSR is correlated with the clinical manifestation of anhidrosis. Absent SSR and orthrostatic hypotension were noted in our patients, indicating that botulism impairs both sudomotor and vasomotor functions.

The site of action of botulinum toxin is the presynaptic terminal. Though the primary abnormality is in transmitter release, a presynaptic defect could involve an abnormality in transmitter synthesis, storage, or release.[1-3] Inasmuch, the levels of plasma norepinephrine in our patients were very low at rest. Ziegler[16] observed that the plasma catecholamine levels are often low in autonomic disorders due to a peripheral nervous lesion. While autonomic disorders in a central nervous lesion, the catecholamine levels are usually within normal range.[17] A postural change from reclining to standing may activate sympathetic activity and increase blood levels of norepinephrine twofold.[6] Such a biochemical change may increase peripheral vessel resistance and heart rate in order to maintain blood pressure. Postural change did not significantly raise the plasma norepinephrine, indicating a peripheral neurogenic mechanism in the orthostatic hypotension of our patients.

Autonomic dysfunction in botulism type B may last more than 7 months.[13] This could explain why the *p* values of DB RRIV of our patients were in the borderline range 6 months after onset. Dysregulation of heart rate and blood pressure is impaired early in some cases, possibly because of the baroreflex dysfunction.[13] Compared with neuromuscular transmission, the autonomic functions of botulism patients had a slower recovery.[13] Our study indicated that the autonomic function of botulism patients could be adequately tested by SSR, RRIV and plasma norepinephrine levels. These tests confirm that botulism involves both sympathetic and parasympathetic pathways.

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