Quantitative Studies of Autonomic Function

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Course Description
Dysfunction of the peripheral and central autonomic nervous system is common in many neurological and general medical diseases. The quantitative assessment of sympathetic and parasympathetic function is essential to confirm the diagnosis of autonomic failure, to provide the basis for follow-up examinations, and potentially to monitor successful treatment. Various procedures have been described as useful tools to quantify autonomic dysfunction. The most important tests evaluate cardiovascular and sudomotor autonomic function. In this review, we therefore focus on standard tests of cardiovascular and sudomotor function such as heart-rate variability at rest and during deep breathing, active standing, and the Valsalva maneuver, and on the sympathetic skin response. These tests are widely used for routine clinical evaluation in patients with peripheral neuropathies. Refined methods of studying heart-rate variability, baroreflex testing, and detailed measures of sweat output are mostly used for research purposes. In this context, we describe the spectral analysis of slow modulation of heart rate or blood pressure, reflecting sympathetic and parasympathetic influences, and consider various approaches to baroreflex testing, the thermoregulatory sweat test, and the quantitative sudomotor axon reflex test. Finally, we discuss microneurography as a technique of direct recording of muscle sympathetic nerve activity.

Intended Audience
This course is intended for Neurologists, Physiatrists, and others who practice neuromuscular, musculoskeletal, and electrodiagnostic medicine with the intent to improve the quality of medical care to patients with muscle and nerve disorders.

Learning Objectives
Upon conclusion of this program, participants should be able to:
1. identify the various procedures described as useful tools to quantify autonomic dysfunction.
2. discuss the spectral analysis of slow modulation of heart rate or blood pressure, reflecting sympathetic and parasympathetic influences, and consider various approaches to baroreflex testing, the thermoregulatory sweat test, and the quantitative sudomotor axon reflex test.
3. recognize microneurography as a technique of direct recording of muscle sympathetic nerve activity.

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INVITED REVIEW

ABSTRACT: Dysfunction of the peripheral and central autonomic nervous system is common in many neurological and general medical diseases. The quantitative assessment of sympathetic and parasympathetic function is essential to confirm the diagnosis of autonomic failure, to provide the basis for follow-up examinations, and potentially to monitor successful treatment. Various procedures have been described as useful tools to quantify autonomic dysfunction. The most important tests evaluate cardiovascular and sudomotor autonomic function. In this review, we therefore focus on standard tests of cardiovascular and sudomotor function such as heart-rate variability at rest and during deep breathing, active standing, and the Valsalva maneuver, and on the sympathetic skin response. These tests are widely used for routine clinical evaluation in patients with peripheral neuropathies. Refined methods of studying heart-rate variability, baroreflex testing, and detailed measures of sweat output are mostly used for research purposes. In this context, we describe the spectral analysis of slow modulation of heart rate or blood pressure, reflecting sympathetic and parasympathetic influences, and consider various approaches to baroreflex testing, the thermoregulatory sweat test, and the quantitative sudomotor axon reflex test. Finally, we discuss microneurography as a technique of direct recording of muscle sympathetic nerve activity.

QUANTITATIVE STUDIES OF AUTONOMIC FUNCTION

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Studies of autonomic function are best considered by the systems being tested and then by reference to whether the tests are routine or research techniques.

CARDIOVASCULAR AUTONOMIC TESTING

Routine Cardiovascular Autonomic Testing: Analysis of Heart-Rate Variability in the Time-Domain. Biosignals such as heart rate or blood pressure can be recorded as time series and show a continuous variability that is altered with autonomic dysfunction. Similar to the electrocardiogram, the variability of heart rate can be assessed under resting conditions and during challenge.23,87 There are various parameters of heart-rate variability.23,84,87 The standard deviation of the heart rate, i.e., the square root of its variance, recorded during an interval of, for example, 5 min, or the coefficient of variation reflects the influence of the parasympathetic and sympathetic system on heart-rate modulation.84,87 The coefficient of variation, i.e., the standard deviation divided by the mean interval between two electrocardiographic R waves (RR interval) during the measuring period, has been introduced for cardiovascular autonomic evaluation as it minimizes the dependence of the standard deviation on resting heart rate. The standard deviation depends on the intrinsic heart rate of the individual patient and moreover is significantly altered by single extrasystoles or stepwise heart-rate changes.29,31 The length of the recorded heart-rate time series also influences the standard deviation.87 Usually, resting heart-rate variability is analyzed from short-term segments of 5-min duration.23

The pNN50 is a parameter that indicates the proportion of differences in consecutive, so-called normal-to-normal RR intervals that are longer than 50 ms. The parameter is independent of long-term...
trends in heart rate and reflects the percentage of such intervals in comparison to the total number of analyzed intervals. The pNN50 is a parameter of parasympathetic activity. Similarly, the root-mean-square of successive differences (RMSSD) reflects parasympathetic activity and does not depend on heart-rate trends. RMSSD is calculated as the square root of the mean squared differences of successive RR intervals. RMSSD is influenced by premature ventricular contractions with long compensatory pauses. It is assumed that this parameter is robust against gradual trends in heart rate over time and is independent of mean heart rate.

We consider the coefficient of variation and the RMSSD the most valuable time-domain parameters for routine evaluation at rest as they provide highly reproducible results and are not influenced by mean resting heart rate.

**Assessment in Research: Frequency-Domain Analysis of Heart-Rate Variability.** Using time-domain analysis, autonomic cardiovascular regulation is often investigated by measuring mean responses of heart rate or blood pressure to external stimuli, e.g., tilting. However, the calculated net changes of blood pressure or heart rate often disregard information on the instantaneous dynamics of autonomic modulation. Analysis of spontaneously occurring fluctuations in autonomic tone by means of frequency-domain (i.e., spectral) analysis might offer a more subtle insight into the physiological mechanisms of autonomic cardiovascular control.

Spectral analysis allows the overall variance of a biosignal to be split into its various underlying frequency components as most biosignals that vary around a mean value can be reconstructed as a sum of sines and cosines at different frequencies. Differences in the magnitudes of the different frequencies or oscillations can be attributed to differences in the neural influences responsible for cardiovascular regulation. The magnitudes of slow underlying frequencies are widely considered indices of sympathetic and parasympathetic tone. Three main components within the slow underlying frequencies have been used in the literature: the very-low-frequency range (VLF ≤ 0.04 Hz), the low-frequency range (LF: 0.04–0.15 Hz) and the high-frequency range (HF: 0.15–0.4 Hz). In recordings of only 5 min, the interpretation of the VLF component is unclear. The fluctuation in heart rate in the LF range is influenced by the baroreceptor system and reflects sympathetic as well as parasympathetic influences, particularly under resting conditions. The LF modulation increases during orthostatic challenge, mental stress, and moderate exercise. Sympathetic as well as parasympathetic outflow may influence the power of low-frequency heart-rate modulation. The heart-rate modulation in the HF range is mainly due to parasympathetic efferent influences and represents respiratory variability. For heart-rate variability, the power of signal modulation is assessed as the area under the spectral density curve in the frequency range considered to reflect sympathetic or parasympathetic activity. If heart-rate variability is measured as beats per minute, the power is given in bpm; if measurements are taken as RR intervals, the power is expressed in ms². The integral under the power spectral density curve is calculated for frequency ranges that have been shown to be influenced by sympathetic and parasympathetic activity using sympatholytic medication or atropinization.

Some authors prefer to calculate the relative value of the LF or HF power components in proportion to the total power in the frequency range from 0.04 to 0.4 Hz. Other authors calculate the ratio between LF and HF power as a parameter of the sympatho-vagal balance. During increased sympathetic activity, the LF–HF ratio will show higher values. During predominance of the parasympathetic system, the ratio will show smaller values.

Various algorithms have been applied to determine the frequencies accounting for the modulation of heart rate and other biosignals by sympathetic or parasympathetic tone. Among the algorithms used for the frequency analysis of biosignals are parametric and nonparametric methods. An important requirement of spectral analysis is the “stationarity” of the signal, a condition easily fulfilled in technical signals but mostly not existing in human physiology. If the mechanisms accounting for the signal modulation in a particular frequency are changing due to physiological changes (for example, during exercise) the significance of a given frequency component for the modulation of the biosignal is no longer well-defined. Moreover, the powers of signal oscillations in frequency bands considered to reflect sympathetic or parasympathetic influence cannot be considered an absolute measure of sympathetic or parasympathetic outflow. Heart rate during exercise will be high due to a predominant sympathetic tone. Yet, the oscillation of sympathetic influences of heart rate might be lower than under resting conditions because of the continuously high sympathetic outflow. Consequently, the power of heart-rate modulation in the frequency range considered to reflect sympathetic
activity will be low and might be misinterpreted as a deficiency of sympathetic outflow, although sympathetic tone is actually high.19,37

Frequency-domain analysis generally requires relatively long, nearly artifact-free recordings. Evaluation of the recorded signals is time consuming and requires specific software and computer techniques. Although the parameters of spectral analysis are useful measures of cardiovascular autonomic function, this technique is therefore mostly restricted to research activities.

**Autonomic Challenge Maneuvers.** Various maneuvers are suited to activate the sympathetic or parasympathetic nervous system.24,50 According to the criteria of the American Diabetes Association, diabetic autonomic neuropathy—the most common autonomic neuropathy in civilized countries—is characterized by both, cardiovascular autonomic dysfunction and impaired sudomotor function. The American Diabetes Association and the American Academy of Neurology have recommended a standard battery for the screening of autonomic dysfunction.14 Tests, primarily analyzing parasympathetic heart-rate control are the Valsalva maneuver, metronomic breathing, and active standing. Active standing can also be used to monitor sympathetic blood pressure control.14

For routine evaluation of cardiovascular autonomic function, we consider the Valsalva maneuver, active standing, and especially metronomic breathing the most valuable tests. The many other tests activating sympathetic or parasympathetic tone include the cold pressor test, the cold face test, squatting, coughing, and mental arithmetic.24,50

**Valsalva Maneuver.** The Valsalva maneuver is known to influence heart rate, but actually evaluates the baroreflex arc and its sympathetic and parasympathetic responses. During the maneuver, the person breathes into a special mouthpiece that is connected to a manometer and maintains an expiratory pressure of 40 mmHg for 15–20 s.3 The maneuver consists of four phases5 (Fig. 1). Phase 1 occurs during the first 2–3 s of the forced expiration and shows a brief decrease in heart rate and increase in blood pressure due to mechanical compression of the aorta. During phase 2, blood pressure first decreases and then increases in the late portion of this phase. The initial decrease in blood pressure activates the baroreflex and results in an increase of sympathetic activity and a consequent increase of peripheral resistance and blood pressure during the late stage of phase 2. Venous return is lowered because of the continuous expiratory strain and reduces cardiac stroke volume, which again results in a baroreflex-mediated tachycardia and peripheral vasoconstriction. Phase 3 describes the first 1–2 s after release of the expiratory strain. There is a passive decline in blood pressure and increase in heart rate. Finally, phase 4 shows a blood pressure overshoot that is due to the persistent increase in peripheral resistance, and a normalization of venous return and stroke volume.37 Mean blood pressure can increase by more than 10 mmHg, but an increase is absent in patients with sympathetic dysfunction.65 The blood pressure increase mediates a baroreflex-induced bradycardia. This bradycardia is a measure of baroreflex buffer capacity and vagal cardiac innervation. Blood pressure changes during phases 2 and 4 reflect sympathetic responses.

The Valsalva ratio is used as an index of the baroreflex-mediated bradycardia and is calculated as the ratio of the highest heart rate during expiration.
and the lowest heart rate during the first 20 s after the expiratory strain. The ratio depends not only on age and gender of the tested person but also on body position, and the duration and intensity of the expiratory strain. The maneuver should be repeated several times to assure reproducibility and reliability of results. When performed carefully and evaluated correctly, the Valsalva maneuver is a helpful tool in the assessment of cardiovascular autonomic function. Low and coworkers established age- and gender-specific normative data. A Valsalva ratio below 1.10 is abnormal; according to some authors, even a ratio below 1.2 is abnormal.

Deep (Metronomic) Breathing. Deep metronomic breathing at a rate of six cycles per minute is probably the most common and reliable test to assess respiratory sinus arrhythmia, with acceleration of heart rate during inspiration and deceleration during expiration under optimized conditions (Fig. 2). There is a decrease of sinus arrhythmia with increasing age.

The central neuronal circuitry involved in inspiration likely inhibits cardiovagal neurons. Changes of the central venous blood volume during respiration also influence heart rate by activation of the Bainbridge reflex while the mechanical modulation of blood pressure activates or inhibits the baroreflex and thus affects heart rate. Moreover, stretch receptors in the lungs and chest walls activate brainstem nuclei and thus modulate the sympathetic and parasympathetic outflow to the heart. Heart-rate modulation during deep breathing depends on parasympathetic cardiac control and is largely reduced with atropinization. A respiratory frequency of six cycles per minute induces maximal heart-rate variability in healthy persons. Low et al. suggest that the average of the five largest consecutive responses in eight respiratory cycles is analyzed. Others prefer to use shorter segments in order to avoid hypocarbia with prolonged hyperventilation. The expiratory–inspiratory difference (E-I difference) or the expiratory–inspiratory ratio (E/I ratio) can be determined from the maximum and minimum heart rate during respiration at six cycles per minute. The values depend not only on age and sex but also on body position, body weight, drugs, and respiratory effort. Single reference values are therefore misleading. E-I differences exceeding 15 heart beats per minute are considered to be abnormal. E-I differences below 10 beats per minute are abnormal in persons below the age of 40 years. Differences below 5 beats per minute are abnormal in persons older than 50 years. The E/I ratio should exceed values of 1.23 in persons younger than 20 years. The E/I ratio should be higher than 1.06 in patients between 76 and 80 years.

Sustained Handgrip Test. Ewing and Clarke introduced the sustained handgrip test as a standard part of a battery used to diagnose diabetic cardiac autonomic neuropathy. The test results are, however, quite variable. First, the subject is asked to press a handgrip dynamometer with full strength. Then, the handgrip should be maintained for 3–5 min at one-third of the maximum contraction strength. The early acceleration of heart rate during the maneuver is due to a withdrawal of vagal activity, whereas the late heart-rate acceleration results from sympathetic activation. Normally, diastolic blood pressure at the end of the effort is at least 16 mmHg higher than before the maneuver. A diastolic blood pressure increase by only 10 mmHg or less is abnormal. Many patients perform a Valsalva maneuver during the handgrip test and consequently bias the test result.

Cold Pressor Test. The cold pressor test (CPT) consists of immersion of one hand and arm in ice cold (0–4°C) water for 40–180 s. The cold stimulus activates afferent pain and temperature fibers from the skin. The impulses pass via the spinothalamic tract...
tract to several brain areas and result in sympathetically mediated heart-rate acceleration, peripheral vasoconstriction, and an increase in blood pressure (Fig. 3). In the first 30 s, blood pressure seems to increase due to a rise of heart rate and cardiac output. Robertson reports an average increase of systolic blood pressure by 20 mmHg and of heart rate by 10 beats per minute (bpm). Vasoconstriction can be recorded by monitoring superficial skin blood flow, e.g., at the digit pulps, using laser Doppler flowmetry. According to Yamamoto et al., peripheral vasoconstriction contributes to blood pressure elevation at a later stage of CPT. With hand immersion into 4°C water, muscle sympathetic nerve activity increased after 30 s and peaked after 60–90 s of cold stimulation. In contrast, Low and coworkers observed a 51% reduction in skin blood flow at the index finger pulp after only 20 s of hand immersion into 0–2°C cold water. These results suggest that responses differ significantly depending on the stimulus duration, water temperature, and whether the hand or arm is immersed in the ice water.

In patients with afferent small-fiber neuropathy or spinothalamic tract dysfunction or with central or efferent sympathetic lesions, such as occur in diabetic or alcoholic autonomic neuropathies, the responses to CPT are diminished or even absent. Reproducibility of CPT results should be assured, although subjects often consider the test unpleasant and are reluctant to participate in a second test run. Therefore, age-matched control data are required for a meaningful interpretation of the results.

**Cold Face Test.** The cold face test (CFT) consists of application of cold compresses (1°C to 2°C) to the forehead and maxillary region of the subject’s face for a period of 60–180 s. Cold stimulation of the forehead and maxillary region activates the peripheral sympathetic and the cardiac parasympathetic nervous system. The combined activation induces a decrease of heart rate as well as a peripheral vasoconstriction with subsequent blood pressure increase (Fig. 4). Bradycardia is the best documented and most easily quantified of the circulatory responses following CFT. Baroreflex activation is probably not essential to induce bradycardia, as the heart rate decrease has been recorded even in the absence of blood pressure changes.

The CFT is a modification of the so-called diving reflex that occurs with immersion of the face in water. The CFT is more comfortable than the diving reflex, but has similar sensitivity towards inducing a cardiovagal response. CFT is noninvasive and can be used to evaluate parasympathetic responses in patients unable to cooperate with other challenge maneuvers. During the CFT, the bradycardia is induced by cold, wet, or noxious stimulation of the face via reflex centers located in the brainstem region. Efferent sympathetic pathways mediate peripheral vasoconstriction and consequent blood pressure increase. Efferent cardiac parasympathetic pathways mediate bradycardia, which can be abolished by atropinization or vagotomy. A disturbance in integrity of the trigeminal-brainstem-vagal reflex arc at any level yields a pathological CFT response with absent or diminished bradycardia. Khurana et al. observed an attenuated cardiovagal response or even minimal tachycardia in response to cold face stimulation in patients with diabetes mellitus, brainstem stroke, or Shy–Drager syndrome.

**Orthostatic Challenge by Head-Up Tilting or Active Standing.** Orthostatic challenge causes an early cardiovascular response that occurs within the first 30 s. During assumption of the upright position, 300–900 ml of blood are redistributed from central blood vessels to the lower extremity. The early circulatory stabilization occurs after 1–2 min of orthostasis. Finally, there is a response to prolonged orthostasis lasting for more than 5 min.

The early phase of stabilization and the adjustments during prolonged orthostasis are similar for active standing and passive tilting. Passive tilting is better suited to assess neurocardiogenic syncope, i.e., to evaluate neuronal control during long-duration orthostatic challenge. Moreover, passive tilting is more suited than active standing if patients have lower-limb weakness or if there is a need to return patients rapidly to the supine position.

Active standing (the so-called Ewing maneuver) is more suited to assess responses during the initial phase of orthostatic challenge. During active standing, there is a contraction of abdominal and leg muscles and a subsequent compression of the resistance and capacitance vessels with increase of the intra-abdominal pressure. Consequently, venous return and cardiac output increase. Yet, this increase does not fully compensate for the decline in total peripheral resistance occurring upon active standing. Therefore, there is a transient reduction in blood pressure during active standing. Muscle contraction activates an exercise reflex that results in a rapid withdrawal of parasympathetic activity and a consecutive heart-rate acceleration within the first 3 s after standing up. The initial reduction in peripheral resistance accounts for a brief drop in blood pressure. The resulting inhibi-
FIGURE 3. Heart rate (HR), blood pressure (BP), and skin blood flow (SBF) during cold pressor test in a control and in a patient with familial dysautonomia (FD). (BPdia, diastolic blood pressure; BPmean, mean blood pressure; BPsys, systolic blood pressure.) In the patient, HR and SBF remain stable, BP increase is reduced and delayed. In the control, SBF decreases initially and shows secondary hyperperfusion (Hunting phenomenon).
tion of the baroreflex induces an ongoing inhibition of cardiac parasympathetic activity as well as an augmentation of sympathetic outflow, both resulting in a secondary, gradual rise in heart rate.91,92 The decreased baroreflex activity and reduced stimulation of cardiopulmonary receptors assures blood pressure recovery after approximately 7 s. Healthy persons might even show a blood pressure overshoot.37
The initial decline and secondary overshoot of systolic and diastolic blood pressure within the first seconds of orthostatic challenge can be recorded by continuous noninvasive blood pressure measurements. According to Wieling et al., an initial drop in systolic blood pressure by more than 40 mmHg or in diastolic blood pressure by more than 25 mmHg is abnormal. After standing for 30 s, heart rate and blood pressure are normalized. Ewing suggested that the ratio is calculated between the highest heart rate, i.e., the shortest RR interval, after approximately 15 heart beats from standing up, and the slowest heart rate, i.e., the longest RR interval, after approximately 30 heart beats from beginning the challenge, as a parameter of orthostatic cardiac response. This 30/15 ratio should be above 1.04, yet values are age-dependent. Responses can also be quantified as the difference between the highest heart rate within 15 s after standing and the baseline heart rate. According to Wieling et al., heart rate should initially increase by more than 20 bpm in young persons (10–14 years old); the increase should not be below 11 bpm in persons aged 75–80 years.

After the initial response to active standing (or passive head-up tilt), there is the early phase of stabilization during 1–2 min of standing. This phase shows an increase in diastolic blood pressure by approximately 10 mmHg and a sympathetic heart-rate increase by 10 bpm. During 5–10 min of active standing or passive head-up tilt, the sympathetic outflow is rather constant and heart rate and blood pressure remain stable.

The initial cardiovascular responses to orthostatic challenge as well as the responses during the first 1–2 min of standing are under neurocardiovascular control. During a prolonged standing phase of more than 5 min, humoral mechanisms contribute to maintaining the blood pressure. Due to an activation of the sympathetic system and the renin-angiotensin-aldosterone system, plasma catecholamine levels increase.

According to Ewing and Clarke, a decline by more than 30 mmHg in systolic blood pressure during active standing is abnormal. Wieling et al. consider a persistent decrease in systolic blood pressure by more than 20 mmHg after 1–2 min of standing or a decrease in diastolic blood pressure by more than 5–10 mmHg as abnormal responses. The consensus committee of the American Autonomic Society and the American Academy of Neurology defined a decrease in systolic blood pressure by at least 20 mmHg or in diastolic blood pressure by at least 10 mmHg within 3 min of active or passive orthostatic challenge as criteria of orthostatic hypotension.

**Head-Up Tilting.** Cardiovascular adaptation to prolonged orthostatic challenge can be tested by means of passive head-up tilting. The cardiovascular changes during head-up tilt are more gradual than during active standing. There is no biphasic response of heart rate and blood pressure, but instead a gradual increase in diastolic pressure and heart rate and no major change in systolic pressure. Consequently, passive orthostatic challenge results in a 5–10 mm increase of mean blood pressure (Fig. 5). The different effects seem to result from the abdominal and lower-limb muscle contractions during active standing and the absence of such contractions during passive tilting. However, muscle activation can only be avoided if the patient is not
brought to the fully upright position. The gravitational stress or hydrostatic effect during orthostasis corresponds to the tilt angle.\(^9\)\(^1\)\(^9\)\(^2\) If the patient is tilted to a 70° angle, the orthostatic challenge is almost identical with the challenge in the upright position (sin 70° = 0.94).\(^9\)\(^1\)\(^9\)\(^2\) To assure that there is no muscle contraction, we only tilt to a 60° angle (sin 60° = 0.87).\(^4\)\(^5\)\(^6\)\(^2\)

It should be noted that the 60° or 70° tilt angle cannot be used to assess mild cardiovagal dysfunction during the initial phase of orthostatic responses.\(^9\)\(^1\)\(^9\)\(^2\) These tilt angles are used to evaluate neurovascular control during the phase of stabilization, i.e., after 1–2 min of challenge and during prolonged 5–10 min, orthostasis.\(^9\)\(^1\)\(^9\)\(^2\) During orthostatic stress, there is an increase of venous pressure in the feet from 5–10 mmHg to approximately 90 mmHg.\(^8\)\(^8\) The higher hydrostatic pressure accounts for progressive fluid transudation from circulating blood into the lower-extremity tissues. The volume shift is better demonstrated during prolonged passive tilting than during active standing with increased skeletal muscle tone that counteracts blood pooling.\(^8\)\(^1\)\(^9\)\(^2\) The reduction of venous return and diastolic cardiac filling upon orthostasis\(^6\)\(^3\)\(^9\)\(^2\) induces a decrease of cardiac output.\(^9\)\(^1\)\(^9\)\(^2\) During prolonged orthostasis, stroke volume can be reduced by 30–40%.\(^9\)\(^1\)\(^9\)\(^2\) Heart rate accelerates by approximately 20% due to baroreflex activation. This heart-rate increase counteracts the cardiac output reduction to some extent.\(^9\)\(^1\)\(^9\)\(^2\) Consequently, the decrease of cardiac output is approximately 20%.\(^9\)\(^1\)\(^9\)\(^2\)

The increase in hydrostatic pressure in the lower-extremity veins also activates the venaarteriolar axon reflex, which increases vascular resistance by up to 40% during standing.\(^3\)^5\(^3\)\(^6\)\(^9\)\(^2\) The venaarteriolar axon reflex further reduces the decrease of cardiac output and slows the loss of fluid into the surrounding tissue.\(^3\)^5\(^3\)\(^6\) In response to orthostatic challenge, there is also a constriction of splanchnic resistance vessels with expulsion of the splanchnic blood reservoir and a consecutive increase of venous return and cardiac filling.\(^9\)\(^1\)\(^9\)\(^2\) This splanchnic vasoconstriction is essential for orthostatic tolerance.\(^9\)\(^2\)

The decrease in blood pressure and cardiac filling upon orthostatic challenge activates the baroreflex which, in turn, inhibits cardiovagal outflow, enhances sympathetic activity, and thus increases heart rate, peripheral vascular resistance, and blood pressure.\(^1\)\(^1\)\(^9\)\(^9\)\(^2\)

**Baroreflex Sensitivity Testing.** Various approaches can be used to test the sensitivity of the most important reflex arc assuring short-term stability of heart rate and blood pressure upon changes of body position.

The so-called sequence method analyzes the relation between fluctuations in heart rate and blood pressure under resting conditions.\(^3\)^0\(^6\)\(^6\) The baroreflex sensitivity is calculated as the slope of the regression between spontaneous sequences of blood pressure increases (or decreases) and simultaneously occurring increases or decreases in the electrocardiographic RR intervals.\(^3\)^0\(^6\)\(^6\) Using this approach, Parati and coworkers found a baroreflex sensitivity of 7.6 ± 2.0 ms/mmHg for blood pressure increases, i.e., positive sequences, and a sensitivity of 6.4 ± 1.5 ms/mmHg for blood pressure decreases, i.e., negative sequences.\(^6\)\(^6\) In contrast to the standard method of phenylephrine injection,\(^7\) the sequence method is noninvasive and does not require patient cooperation. However, the sequence method only evaluates the baroreflex sensitivity without challenge and under resting conditions and therefore only shows a small portion of the baroreflex curve.\(^3\)\(^7\)

**Spectral analysis of heart-rate and blood pressure variability** can also provide a measure of spontaneous baroreflex sensitivity.\(^3\)\(^7\) Instead of comparing the direct changes of blood pressure and consecutive changes in heart rate, the changes in sympathetically mediated spectral components of blood pressure modulation and the consecutive changes in the frequencies reflecting sympathetic heart-rate modulation are used to describe baroreflex sensitivity.\(^3\)\(^7\) With this method, baroreflex sensitivity is assessed by analyzing the relationship between spontaneous, sympathetically mediated fluctuations of the blood pressure, reflecting the input activity of the baroreflex, and corresponding heart-rate fluctuations, reflecting the reflex output activity. The amplification between input and output of the reflex is an index of baroreflex sensitivity. Mathematically, this amplification equals the so-called gain of the transfer function between the oscillations of blood pressure and heart rate in the LF range, provided there is sufficient coherence, i.e., a stable and reliable relationship between both biosignals.

Robbe and coworkers demonstrated that this baroreflex gain provides an index of baroreflex sensitivity that is closely correlated with the results of the phenylephrine method.\(^7\)\(^1\) The values assessed by calculating the gain of the transfer function between low-frequency oscillations of blood pressure and low-frequency oscillations of the RR interval (18.1 ± 8.9 ms/mmHg) are similar to the baroreflex sensitivity assessed by calculating heart-rate deceleration in response to phenylephrine-induced blood pressure increases (16.2 ± 7.3 ms/mmHg).\(^7\)\(^3\) Both the se-
Quantitative Studies of Autonomic Function

The sequence method and the calculation of the low-frequency transfer function gain provide noninvasive measures of baroreflex sensitivity, but only evaluate the reflex under resting conditions; they do not assess blood pressure modulation in response to baroreflex activation. Moreover, the gain of the transfer function between systolic blood pressure and heart rate may depend on variables such as mechanical, respiratory, or movement-induced changes in blood pressure or heart rate.

Pharmacological baroreflex testing is the standard approach for measuring baroreflex sensitivity that was introduced by Smyth et al. This so-called Oxford-method assesses heart-rate changes in response to pharmacological blood pressure stimulation. Rapid infusion of phenylephrine increases blood pressure and thereby activates the baroreflex and generates a bradycardia. The baroreflex sensitivity is calculated as the slope of the linear regression between beat-to-beat systolic blood pressure values and values of the RR interval. Smyth and colleagues calculated the baroreflex sensitivity between 2 and 15.5 ms/mmHg in awake subjects and between 4.5 and 28.9 ms/mmHg in sleeping persons.

The use of cardiovascular depressor or pressor drugs better characterizes the baroreflex sensitivity over a wide range of arterial pressures. The application of ramps of increasing or decreasing arterial pulses allows the entire sigmoid baroreflex curve to be described. However, the method must be avoided in patients with arterial hypertension. Moreover, it defines heart-rate responses but not blood pressure responses to baroreflex activation.

In 1957, Ernsting and Parry described baroreflex stimulation by means of a neck chamber. The method was further modified by Eckberg and coworkers and Bernardi et al. The carotid sinus and baroreceptors can be mechanically deformed by applying a positive or negative pressure to the neck. Nowadays, a moulded lead collar is used to cover the anterior part of the neck (Fig. 6), and a positive or negative atmospheric pressure is applied to the reservoir between the neck and the collar. A modified vacuum cleaner is normally used to induce a negative pressure within the neck collar. To avoid interference with the respiratory sinus arrhythmia, stepwise pressure changes should be applied while the patient holds his or her breath at the end of normal expiration. The negative pressure distends the neck tissue including the baroreceptors and carotid artery, similar to the carotid artery and baroreceptor distension occurring with a blood pressure increase. The distending pressure is compared to baroreflex-mediated changes in the electrocardiographic RR intervals.

Thermoregulatory Sweat Test. Central and peripheral sudomotor function can be tested by means of the thermoregulatory sweat test (TST). In order to identify skin areas with sweat production, an indicator powder is required. The humidity of the sweat changes the color of this powder. Consequently, the patient’s entire skin surface has to be dusted with the powder, and the patient remains in a sweat chamber that is used to heat the body core temperature, usually with infrared heaters. The sweat chamber must be temperature- and humidity-controlled. Air temperature should be at 45–50°C, and humidity at 35%–40%.

FIGURE 6. Activation of carotid baroreceptors by means of “neck suction” using a moldable lead collar. Sympathetic and parasympathetic baroreflex responses are induced by applying different levels of positive and negative pressures to the neck and the baroreceptors.

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approximately 45–65 min. There must be an increase in oral temperature by at least 1.0°C or to 38°C, but not to above 38.5°C. Anatomi-cal drawings or photographic recordings can be used to document areas of absent sweating, reduced sweating, or hyperhidrosis. Areas of abnormal sweating are calculated as a percentage of the anterior body surface. The test can be used to assess sudomotor dysfunction in various diseases such as primary autonomic failure or central and peripheral secondary autonomic dysfunction, for example in neuropathies, myelopathy, or skin or sweat gland disorders. Global anhidrosis can be seen in primary autonomic failure. The TST is abnormal in patients with central sudomotor dysfunction whereas postganglionic sweating—as assessed with the quantitative sudomotor axon reflex test—usually shows preserved function of the postganglionic sudomotor fibers and sweat glands in these patients. However, the TST can also be used to monitor peripheral sudomotor function and assess severity and progression of anhidrosis in an autonomic neuropathy, e.g., in diabetic patients.

Quantitative Sudomotor Axon Reflex Test. The quantitative sudomotor axon reflex test (QSART) assesses the postganglionic sudomotor nerve fibers and the sweat glands in localized areas of the skin. Acetylcholine (ACh) is brought into the skin by means of iontophoresis, and then binds to the muscarinic receptors on the eccrine sweat glands. This results in a first, direct sweat response. ACh, however, also binds to nicotinic receptors of the postganglionic sudomotor axons and induces antidromic C-fiber activation. The impulse travels along the C-fibers and reaches branching points from where it activates sweat glands by orthodromic activation of the branching C-fiber nerve terminals. The activation of C-fiber branches finally evokes the indirect, axon reflex–mediated sweat gland response next to the site of initial iontophoretic stimulation. The sweat response is terminated by the cleavage of ACh into acetate and choline by acetylcholinesterase. A multicompartmental sweat cell is used to measure the sweat production. The cell contains a compartment—usually the inner compartment—that is filled with 10% acetylcholine. There is also a second compartment—usually the outer compartment—that takes up the humidity from the axon-induced sweat production. The sweat in this compartment is evaporated and carried to a hygrometer by means of a nitrogen gas flow. The hygrometer continuously monitors the relative humidity. During the test, the skin temperature should be kept at approximately 34.5°C to optimize the sweat response. The iontophoresis of acetylcholine is induced by a constant, 2-mA anodal current from the stimulus compartment to the skin. The cathode is placed on the skin near to the stimulation site.

Sweat responses are usually recorded at baseline, during 5 min of iontophoresis, and 5–10 min after iontophoresis to observe the return of sweat output to baseline. Normally, there is a 1–2 min latency between the onset of iontophoresis and reflex-mediated sweating. The area under the curve describing the sweat output is a parameter of the axon reflex–mediated sweat response (Fig. 7). The tests provides highly reproducible results and detects postganglionic sudomotor dysfunction. However, QSART testing can only evaluate the sweat output at the site where the multicompartmental sweat chamber covers the skin. The technique does not allow a general statement on postganglionic sudomotor function in an individual patient. Due to the dying-back pathology of many peripheral neuropathies, QSART testing may be useful to map the stocking- or glove-like distribution of postganglionic sudomotor dysfunction. The proximal dorsal foot, the lateral proximal calf, the distal medial leg, and the middle forearm can be used as standard recording sites. Low reported various types of QSART responses. In painful neuropathies or in patients with reflex sympathetic dystrophy, there are persistent QSART responses that might result from enhanced sympato-sympathetic reflexes. The QSART shows abnormal responses in patients with postganglionic sudomotor fiber dysfunction, e.g., in various neuropathies (Fig. 8). In preganglionic, central disorders such as multisystem atrophy or spinal cord injuries, QSART responses are usually normal.
Sympathetic Skin Response. Various authors recommend measurements of the sympathetic skin response (SSR) to evaluate sudomotor function. However, the results of sympathetic skin response testing should be interpreted carefully.80 Any arousal stimulus can be used to activate sympathetic sudomotor nerve fibers and thereby change skin resistance.2,4 The central pathways mediating the arousal response and sudomotor activation are not known completely.94 The SSR is usually recorded from the palms of the hands and soles of the feet, with the reference electrodes on the dorsum of the hands and feet. The low-frequency filter should be as low as possible, e.g., at 0.1 Hz, and the high-frequency filter should be at 30 Hz.48 SSR can be evoked by various types of stimulation, such as electrical, acoustic, or inspiratory gasp stimuli. As the response habituates, stimuli must be delivered at randomized intervals and with an increasing stimulus intensity.80 The response is rather variable and there are no clear criteria regarding abnormal responses.80 Yokota and coworkers consider the SSR abnormal if amplitudes between the left and right side differ by at least 50% or if one of four limb responses is absent.94 Although the SSR is easy to perform,53,40 the technique is not very sensitive in the early detection of small-fiber neuropathy and in detecting autonomic sudomotor dysfunction.80

CONCLUSION

Many tests have been used to quantify autonomic dysfunction and are described in standard textbooks. All of the tests show age-dependent results and often significant differences between women and men.50 There are concerns that the variability of procedures used for autonomic testing is excessive, but study designs often fail to take into account the need to standardize procedures to assure reproducibility of the results. The results of a Valsalva maneuver will differ greatly, not only if the level or duration

MICRONEUROGRAPHY

Whereas measurements of cardiovascular modulation and sudomotor function are indirect means to monitor sympathetic and parasympathetic activity, microneurography is used for direct recordings of bursts of efferent muscle sympathetic nerve activity (MSNA).15,89 However, due to its invasiveness and the time-consuming nature of the procedure, MSNA may be more relevant for research purposes than for routine evaluations of autonomic function. In addition, abnormal findings at one site do not necessarily allow extrapolation to suggest generalized autonomic dysfunction. Still, the technique is probably the best and most direct measure of sympathetic activity. Since MSNA reflects vasoconstrictor activity to intramuscular vessels, it is closely related to intramuscular vasoconstriction and vascular resistance.89 Moreover, a close correlation exists between plasma norepinephrine levels and MSNA.71 MSNA can be recorded from peripheral nerves such as the median, tibial, or peroneal nerve.89 Most often, the peroneal nerve is examined. A recording electrode is inserted into a single nerve fascicle while the reference electrode is inserted subcutaneously.53,89 Filters are set at 700 Hz and 2 kHz, the signal is preamplified and rectified, and the mean voltage or voltage area is assessed.89 The number of bursts per minute is multiplied by the mean burst amplitude or mean voltage area to measure sympathetic nerve activity.65 The MSNA bursts occur with an individually stable latency from the electrocardiographic R-wave, indicating that the MSNA bursts are related to an inhibitory effect of systole on the arterial baroreceptors.77,89 MSNA activity increases after baroreceptor denervation and the relation to the cardiac rhythm disappears.15 MSNA is inhibited during baroreflex activation.15 In contrast, MSNA increases with unloading of baroreceptors, for example during orthostatic challenge by passive tilting, or during the blood pressure decrease in phase 2 of the Valsalva maneuver.63,89

FIGURE 8. Normal quantitative sudomotor axon reflex test (QSART) in a healthy control (A) and abnormal QSART in a patient with dysfunction of the postganglionic sympathetic sudomotor axon due to small-fiber neuropathy (B). In contrast to the patient, sweating increases during mental arithmetic (1), and acoustic stimulation (2), in the control prior to acetylcholine iontophoresis (3).
of expiratory strain varies inter- or intra-individually, but also if the test is carried out in supine, sitting, or standing position.\textsuperscript{3,7,10,63} Similarly, results vary if one test is performed early in the morning and another during the late afternoon.\textsuperscript{3,7,63} Room temperature and humidity, and disturbances in background noise will affect the results of autonomic testing.\textsuperscript{3,7,63} Spectral analysis yields results that depend on the algorithm, the length of the analyzed signal epoch, and the stationarity of the analyzed biosignals.\textsuperscript{3,7,63} Frequently, a compromise has to be found between the need for long enough signal epochs and the requirement of stationarity. Individual conditions such as the level of stress and exhaustion or relaxation might also vary from one examination to the next and thus influence the test results. However, the quality and reproducibility of autonomic test results will increase significantly and fulfill the requirements needed for serial examinations if the many different variables influencing autonomic responses are first identified, taken into consideration, and then kept constant, and if testing is highly standardized.

REFERENCES


