

TESTS FOR DYSAUTONOMIAS

There are many tests that can be used to evaluate patients with known or suspected dysautonomias.

Each type of test has advantages and disadvantages. Most centers that carry out autonomic function testing use more than one type of test. None uses all the tests described in this section.

OVERVIEW OF AUTONOMIC FUNCTION TESTS

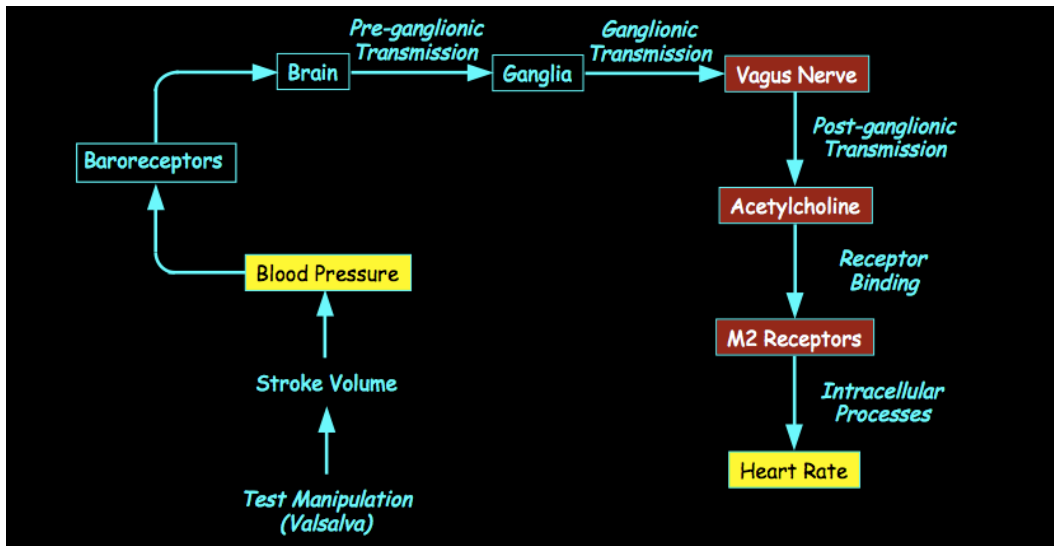
The most important autonomic function test is the medical history.

In the United States, payment by third party payers for management of patients with dysautonomias is based mainly on procedures, even though it is the autonomic history that is most important. Autonomic history-taking can't be done well in a brief clinic visit—a growing problem for community based physicians.

Tests for dysautonomias can be divided into physiological, neuropharmacologic, neurochemical, neuroimaging, and genetic.

Physiological tests involve measurements of a body function in response to a manipulation such as standing, tilt table-testing, or a change in room temperature.

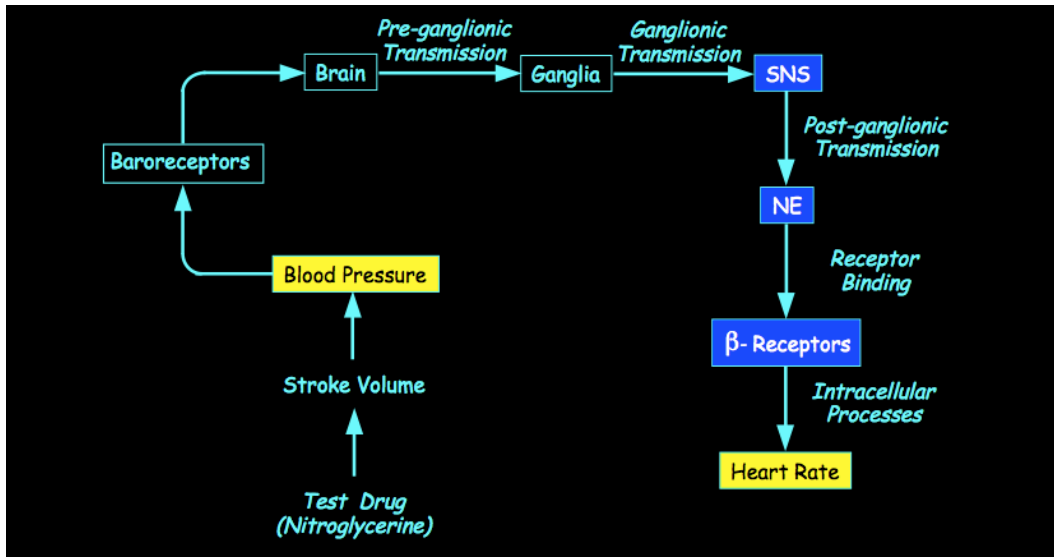
There are always several steps between the brain's directing changes in nerve traffic in the autonomic nervous system and the physiological measures that are chosen to track the autonomic changes. Because of this indirectness, results of physiological tests can be difficult to interpret or may not identify a problem accurately.



Physiological tests involve manipulation of a body function such as blood pressure and measurement of a different body function such as pulse rate.

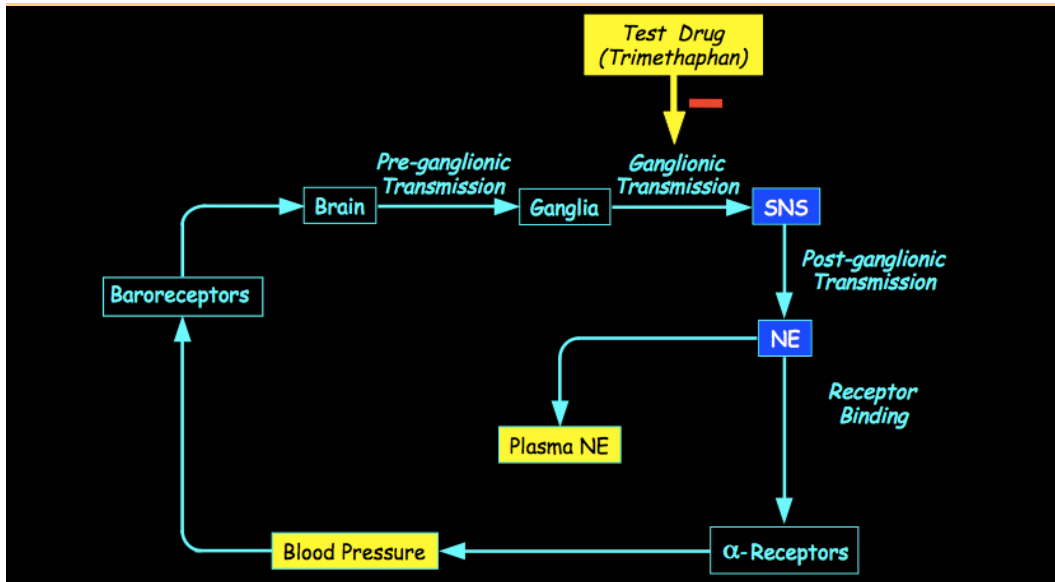
Neuropharmacologic tests involve giving a drug and measuring its immediate effects.

Neuropharmacologic tests of the autonomic nervous system involve measuring effects of drugs, usually on a physiological measure but sometimes on levels a biochemical such as norepinephrine. There always is at least some risk of side effects of the test drugs. In addition, test drugs can interact with drugs the patient is on to treat the disease or with other conditions the patient has.



Neuropharmacologic tests involve drugs that affect the autonomic nervous system.

Sometimes results of neuropharmacologic tests can be as difficult to interpret as those of physiologic tests. For instance, a neuropharmacologic test of the role of the sympathetic nervous system in a person's high blood pressure might include measuring the effects of a drug that blocks sympathetic nerve traffic on blood pressure, because a large fall in blood pressure would suggest an important role of the sympathetic nervous system in keeping the blood pressure high. But if blocking the sympathetic nerve traffic activated another system compensatorily that also increases blood pressure, then the sympathetic blocking drug might not decrease the pressure, and the doctor might mistakenly think that the sympathetic nervous



Neurochemical tests involve measuring chemicals indicating functions of the autonomic nervous system, such as plasma norepinephrine. Physiological and neurochemical measures can be assessed simultaneously.

system wasn't involved with the patient's high blood pressure.

Neurochemical tests involve measuring levels of body chemicals, such as the catecholamines, norepinephrine and adrenaline, either under resting conditions or in response to physiological or neuropharmacologic manipulations. Several factors influence plasma norepinephrine levels, besides release from sympathetic nerves.

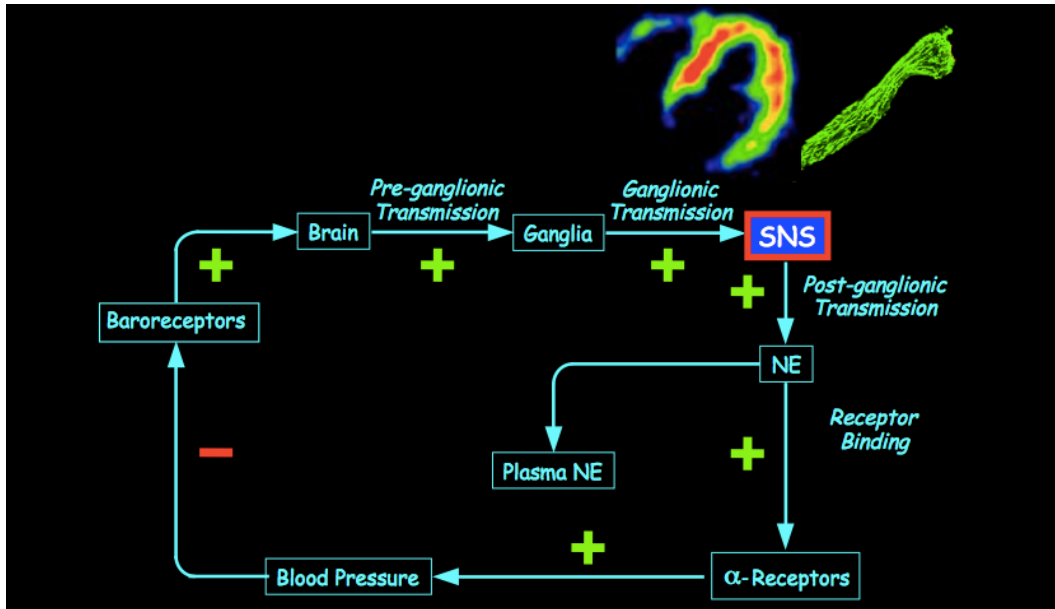
Neurochemical tests can be done on blood samples that are drawn

while the patient is at rest lying down, during a physiological manipulation such as exercise or tilting on a tilt-table, or during a neuropharmacologic manipulation such as blockade of sympathetic nerve traffic by a drug. Neurochemical tests themselves are safe, but the type of body fluid sampling, such as arterial blood sampling or cerebrospinal fluid sampling after a lumbar puncture, can involve some risk. Relatively few centers have a clinical neurochemistry laboratory to carry out the assays. The results can be affected importantly by dietary constituents, drugs, or dietary supplements the patient is taking and by the exact conditions at the time of sampling.

There is no chemical test of parasympathetic nervous system activity. This is because acetylcholine, the chemical messenger of the parasympathetic nervous system, is broken down by an enzyme almost as soon as acetylcholine enters body fluids such as the plasma.

Neurochemical testing to examine activity of the sympathetic noradrenergic system based on plasma norepinephrine levels can be difficult to interpret. Those levels are determined not only by the rate of entry of norepinephrine into the plasma but also the rate of removal (clearance) of norepinephrine from the plasma. In addition, plasma norepinephrine levels are determined by a variety of processes that take place within the sympathetic nerves.

Neurochemical testing by plasma norepinephrine levels requires a



Neuroimaging tests involve seeing parts of the nervous system, such as sympathetic nerves supplying the heart in PET scans or supplying arrector pili muscle in skin biopsies.

carefully controlled testing situation and expert technical analysis and interpretation. Few clinical laboratories measure plasma levels of catecholamines such as norepinephrine and adrenaline, from the point of view of diagnosis or management of dysautonomias, and laboratories vary in the validity of the assay methods they use.

Some blood tests involve measuring levels not of neurochemicals but of factors in the circulation that affect the functioning of one or more components of the autonomic nervous system. For instance, there is an uncommon form of dysautonomia in which there is a high titer of

an antibody to the nicotinic receptor that is required for relaying signals in the ganglia. The associated conditions have been called autoimmune autonomic neuropathy or autoimmune autonomic ganglionopathy.

Neuroimaging tests involve actually visualizing parts of the autonomic nervous system, such as the sympathetic nerves in the heart.

Neuroimaging tests, which are relatively new, involve actually visualizing the autonomic nerve supply in body organs. As yet there is no accepted neuroimaging test to visualize parasympathetic nerves. Sympathetic neuroimaging is done in few centers, and although this type of testing can produce striking images of the sympathetic innervation of the heart, this provides mainly anatomic information about whether sympathetic nerves are present. It is still unclear whether sympathetic neuroimaging can provide information about whether those nerves are functioning normally or not.

Neuroimaging can also be used to identify brain diseases that are associated with dysautonomias. For instance, different types of scans can identify the loss of nerve terminals that contain dopamine in the brain in Parkinson disease, or identify abnormalities of brain structures that regulate the autonomic nervous system.

Genetic tests involve some ethical issues, such as patient

confidentiality and whether an individual wishes to know the test result, if there is no way to prevent the disease. Researchers may be reluctant to provide results of genetic tests, if the laboratory is not certified to do diagnostic testing.

Genetic tests involve analyses of genetic material (DNA) for abnormalities of specific genes that produce or predispose to the development of particular diseases.

Now we will go over in more detail the different forms of clinical testing of the autonomic nervous system.

THE MOST IMPORTANT TEST OF ALL

The most important test in the evaluation of dysautonomia is the medical history.

Symptoms are feelings that the patient reports to the doctor as part of the medical history. Signs are medical findings that a doctor detects during a physical examination.

The medical history consists of several parts. These include the Chief Complaint, the History of the Present Illness (HPI), the Past History, the Family History, the Personal and Social History, and the Review of Systems (ROS). Each of these parts is important for diagnosing and managing dysautonomias, but the key component is the HPI.

The HPI is basically narrative of the condition. It is best to obtain the HPI from the patient directly. There are records to review of hospitalizations, test results, and previous accounts of the medical history and physical examination (HPE). These are all subject to mistakes and often are uninformative. The patient's story of his or her symptoms, especially with the help of family or significant others, is at least as likely to be correct and informative. Unfortunately, this key aspect of the medical encounter is not reimbursed adequately considering its importance and the time and effort involved.

Chief Complaint: In a few words, what's the main problem bothering you that brings you here today?

HPI: When was the last time you felt completely healthy?

What was the first thing that went wrong?

What happened next?

Have you noticed anything that makes the problem worse or better?

What treatments have been tried, and how did you respond?

Autonomic Review of Systems:

Who does your shopping? Are you able to tolerate **standing**, exercise, heat, a large meal?

Do you **sweat** like other people?

Do you make **spit** like other people?

Have you noticed any problems with **urination**?

Have you noticed any problems with **bowel movements**?

Have you noticed any problems with **sexual function**?

Some aspects of the screening autonomic history

I take what the patient says as gospel. The patient knows best how he or she feels, and in my experience patients always tell the truth.

Taking the medical history, especially the HPI, is a skill that must be honed by learning and experience, ideally under the direct supervision of a mentor.

A complete listing of all prescribed medications, over-the-counter medications, herbal remedies, and dietary supplements is a key part of the medical history, not only because these can affect autonomic function but also because they can interact to produce unexpected,

serious adverse events.

For example, I know of a patient with multiple system atrophy (MSA) who first came to medical attention because of paroxysmal high blood pressure evoked by taking *ma huang* tea. The active ingredient in the tea was ephedrine, an amphetamine, and the drug increased delivery of norepinephrine to its receptors. This caused the blood pressure to increase, and because of arterial baroreflex failure as part of the MSA the increase in blood pressure was not buffered by the baroreflex. The patient went to the emergency room, where his blood pressure was so high the physicians thought he had a stroke. Although *ma huang* is no longer sold as a dietary supplement in the US, yohimbe bark is. Yohimbine increases norepinephrine release and in a patient with baroreflex failure could result in paroxysmal hypertension.

Timing is Everything

In obtaining the history of the present illness (HPI), one of the most important skills a clinician can acquire is the ability to get the sequence right.

I usually start by asking the patient, “When was the last time you felt perfectly healthy?” It’s amazing learning the answers, which can range from “I’ve never been healthy” to “I was fine until...” such and such a date to “It was such a gradual thing, I don’t know.”

Some dysautonomias develop in a rather stereotypical sequence. An

example is the cerebellar form of multiple system atrophy (MSA-C) in a man. Men with MSA-C typically relate that the first thing to go wrong, in retrospect, was erectile failure. In my opinion, in a man with central neurodegeneration and orthostatic hypotension, the absence of erectile failure as an early finding rules out MSA-C. The erectile failure is followed by urinary problems—especially urinary retention, eventually to the point of requiring self-catheterization. Then come slurred speech, a wide-based, unsteady gait “like a drunken sailor,” and lightheadedness when standing.

In obtaining the details about symptoms of dysautonomias, it is also important to determine which situations make things worse and which make things better. For instance, patients with neurogenic orthostatic hypotension often relate that their symptoms are worst in the morning, upon heat exposure, after eating a large meal, or after exercise.

Because of associations of autonomic failure with non-motor aspects of Lewy body diseases such as Parkinson disease and pure autonomic failure, it is important to ask about whether the patient smells things like other people, sees things like other people, and has any problems with sleep. The clinician is looking for evidence of olfactory dysfunction, visual hallucinations, and dream enactment behavior.

In patients with possible postural tachycardia syndrome (POTS), it is valuable to ask about whether the patient has “double-jointedness” or stretchy skin, since these can be clues to the existence of Ehlers-

Danos syndrome. Later you will learn about the “coat hanger sign” and the “water bottle sign” in dysautonomias like POTS. Again, the sequence of events can be very informative. Subacute development of orthostatic intolerance after a viral illness suggests an autoimmune component of the pathophysiology, whereas a history of frequent fainting or “seizures” since childhood points more to a congenital, genetic component. In the evaluation of a patient with POTS, which occurs mainly in relatively young women, it is always important to ask, in a private setting, about emotional, physical, or sexual abuse in childhood. These can have long-term consequences in terms of chronic fatigue, altered memory or concentration, and panic or anxiety.

In a patient with labile blood pressure and orthostatic intolerance, a remote history of irradiation of the neck brings to mind the possibility of arterial baroreflex failure due to accelerated arteriosclerosis of the carotid sinus area.

Symptoms & Signs of Dysautonomias

Altered functions of each of the components of the autonomic nervous system result in particular symptoms and signs.

Sympathetic Noradrenergic System (SNS) Failure

Orthostatic intolerance & hypotension

Post-prandial lightheadedness & hypotension

Heat intolerance & hypotension

Fatigue

Tendency to slow pulse rate during exercise

“Coat hanger” pain

Droopy eyelids (ptosis)

Decreased ability to ejaculate

Tendency to constricted pupils

No goosebumps

Symptoms and signs of SNS failure

Failure of the sympathetic noradrenergic system (SNS) manifests as orthostatic hypotension, meaning a persistent, consistent fall in blood pressure each time the patient stands. Orthostatic hypotension can produce symptoms such as lightheadedness, dizziness, faintness, visual changes, and muscle weakness. Collectively these symptoms are termed “orthostatic intolerance.” Orthostatic hypotension can also occur without producing symptoms.

Orthostatic hypotension is a cardinal sign of SNS failure.

Sympathetic Noradrenergic System (SNS) Hyperactivity

Pallor

Tendency to high blood pressure

Tendency to fast pulse rate

Trembling

Bristling hair (piloerection)

Increased production of thick saliva

Tendency to dilated pupils (mydriasis)

Symptoms and signs of SNS hyperactivity

Orthostatic hypotension often is accompanied by post-prandial lightheadedness and hypotension. “Post-prandial” means after eating a meal. In patients with SNS failure, heat exposure also can decrease the blood pressure.

SNS hyperactivity produces pallor, due to constriction of blood vessels in the skin. Blood pressure tends to increase.

PNS hyperactivity results in a tendency to slow pulse rate, nausea, and gastrointestinal upset.

The sympathetic cholinergic system (SCS) is the main part of the

Parasympathetic Nervous System (PNS) Failure

Dry mouth (decreased watery saliva)

Constipation

Dry eyes

Urinary retention

Tendency to fast pulse rate with low variability

Slow gastrointestinal transit

Erectile failure

Symptoms and signs of PNS failure

autonomic nervous system mediating sweating. SNS failure manifests as decreased sweating.

The main symptoms of parasympathetic nervous system (PNS) failure are dry mouth and constipation.

Adrenaline, the main chemical messenger of the sympathetic adrenergic system (SAS), is a hormone, and as adrenaline is distributed by the bloodstream to all the organs (with the exception of most of the central nervous system). Adrenaline injection produces characteristic symptoms, including pallor, increased sweating, cardiovascular stimulation, dilated pupils, and increased blood glucose levels. Adrenaline exerts well-known anti-fatigue effects and

Parasympathetic Nervous System (PNS) Hyperactivity

Increased salivation

Tendency to slow pulse rate or heart block

Nausea & vomiting

Diarrhea or fast gastrointestinal transit

Tendency to constricted pupils (miosis)

Urge to urinate (increased bladder motility)

Increased stomach acid secretion

Symptoms and signs of PNS hyperactivity

tends to increase the intensity of emotional experiences.

SAS failure, on the other hand, produces relatively few symptoms or signs—perhaps a tendency to fatigue or to hypoglycemia.

Sympathetic Cholinergic System (SCS) Failure

Decreased sweating

Sympathetic Cholinergic System (SCS) Hyperactivity

Increased sweating

Altered SCS function involves changes in sweating.

Sympathetic Adrenergic System (SAS) Hyperactivity

Pallor

Increased sweating

Increased heart rate & contractility

Increased systolic & pulse pressures

Dilated pupils (mydriasis)

Increased blood glucose (hyperglycemia)

Decreased gastrointestinal transit (functional ileus)

Tendency to increased emotional intensity, anti-fatigue

Tendency to decreased serum potassium (hypokalemia)

Tendency to decreased bleeding time

Tendency to increased core temperature

SAS hyperactivity produces many symptoms and signs.

Sympathetic Adrenergic System (SAS) Failure

Tendency to hypoglycemia

Tendency to fatigue

SAS failure produces few symptoms or signs.

Composite Autonomic Symptom Score (COMPASS)

Over the years, progressively more refined “composite” autonomic symptom scores (COMPASS) have been introduced. The “COMPASS 31” scale contains a total of 31 questions in 6 domains, yielding an overall autonomic symptom score from 0 to 100. The domains are orthostatic intolerance (4 questions), vasomotor (3 questions), secretomotor (4 questions), pupillomotor (5 questions), bladder (3 questions), and gastrointestinal (including diarrhea, constipation, and gastroparesis, 12 questions). Erectile dysfunction is not included, since this is gender specific. For each question there is a numeric rating based on factors such as site, consistency, severity, frequency, or trends.

Here are the topics and simplified questions of the COMPASS 31:

1. *Orthostatic intolerance*: In the past year, have you ever felt faint, dizzy, “goofy”, or had difficulty thinking soon after standing up from a sitting or lying position? If so, how frequently? How severe are these feelings or symptoms? Have they changed?

2. *Vasomotor*: In the past year, have you ever noticed color changes in your skin, such as red, white, or purple? If so, which body parts are affected? Have these symptoms changed?

3. *Secretomotor*: In the past 5 years, what changes, if any, have occurred in your general body sweating? Do your eyes feel excessively dry? Does your mouth feel excessively dry? For the

symptom of dry eyes or dry mouth that you have had for the longest period of time, has this symptom changed over time?

4. *Gastrointestinal:* In the past year, have you noticed any changes in how quickly you get full when eating a meal? Have you felt excessively full or persistently full (bloated feeling) after a meal? Vomited after a meal? Had cramping or colicky abdominal pain? Bouts of diarrhea? If so, how frequently? How severe are the episodes? Have they changed? In the past year, have you been constipated? If so, how frequently? How severe are the episodes? Have they changed?

5. *Bladder:* In the past year, have you ever lost control of your bladder function? If so, how frequently? Have you had trouble completely emptying your bladder? If so, how frequently?

6. *Pupillomotor:* In the past year, without sunglasses or tinted glasses, has bright light bothered your eyes? If so, how frequently: How severe is this sensitivity to bright light? Have you had trouble focusing your eyes? How frequent is the problem? How severe is the problem? Is the problem with light sensitivity or focusing changing?

While internally consistent statistically and useful for research purposes, composite scoring of autonomic symptoms is incomplete from the point of view of the diagnostic interview as applied to dysautonomias. For instance, within the “orthostatic intolerance”

domain, time of day, relationships with meals, exercise, and heat exposure, associated symptoms such as the coat hanger phenomenon, chronic fatigue, chronic pain, and “brain” fog all should be considered. Within the “bladder” domain, a report of urinary retention and the need for self-catheterization is extremely important for differentiating the parkinsonian form of multiple system atrophy (MSA-P) from Parkinson disease with orthostatic hypotension (PD+OH). Urinary retention strongly favors MSA-P over PD+OH. The lack of inclusion of erectile dysfunction in men is rather glaring.

The COMPASS approach does not take into account the syndromic nature of particular forms of dysautonomia. For instance, in an elderly patient with Parkinsonism, it is highly relevant to ask about olfactory dysfunction, since anosmia (lack of sense of smell) is common in PD+OH; about cognitive function, since dementia is more commonly associated with PD+OH than with MSA-P; about speech, since slurred speech favors MSA-P over PD+OH; and about breathing, since stridor favors MSA-P over PD+OH. In a young women with orthostatic intolerance, asking about double-jointedness and stretchy skin may uncover Ehlers-Danlos syndrome. In a patient with labile hypertension, the past history may disclose a remote history of neck irradiation, raising the possibility of arterial baroreflex failure from carotid arteriosclerosis.

Perhaps most importantly, the COMPASS approach does not take into account the sequence of symptoms, the chronology that is the essence

of the history of the present illness (HPI). For instance, in a man with central neurodegeneration, the lack of early erectile function excludes MSA. The checklist concerns only events within the past year (except for 5 years for secretomotor). In contrast, the non-directed approach to the HPI starts with a question like, “What was the first thing you noticed that went wrong?”

My screening questions generally query each of the components of the autonomic nervous system. The questions are designed not to be leading. For instance, about sympathetic cholinergic function, I ask, “Do you sweat like other people?” About sympathetic noradrenergic function, I ask, “Are you able to tolerate standing still?” About parasympathetic cholinergic function, I ask, “Are you able to make spit and tears like other people?” Have you noticed anything different about how your GI system is working? Have you noticed anything different about your urination? In a man I also ask, “Are you able to have an erection and ejaculate?” As noted above, depending on the specific conditions I have in mind I also ask questions related to dysautonomia syndromes.

A Pain in the Neck

In patients with orthostatic intolerance or orthostatic hypotension, standing upright can result in an annoying pain in the back of the neck and along the shoulders. Because of the distribution of the



The “coat hanger phenomenon” refers to pain in the back of the neck during standing.

discomfort, this is sometimes referred to as the coat hanger sign or coat hanger phenomenon.

The exact mechanism of the coat hanger phenomenon is unknown. I think of it as a kind of cramp when the anti-gravity muscles holding up the head receive too little blood flow. These muscles are active all the time, which means they are using up oxygen all the time that is delivered to them in the arterial blood. If the blood flow falls to below a certain rate, then products of metabolism that cause pain can build up.

Who Does Your Shopping?

Most patients with orthostatic intolerance are women. At the risk of seeming chauvinistic, my main screening question for a woman referred for orthostatic intolerance is, “Who does your shopping?”

If the answer is, “I do. I love to shop,” then that is the end of my line of questioning. A positive answer is something like, “Well not me.” When I ask, “Why not?” the answer I’m looking for is, “Because I can’t tolerate standing still on line. I start to feel faint or lightheaded or weak, or I have to twist my legs like a pretzel, or I have to sit down.”

Pretzel Legs and the Water Bottle Sign

I remember well the first patient I saw with pure autonomic failure (PAF), a rare disease manifesting with orthostatic hypotension due to loss of sympathetic noradrenergic nerves. She was sitting in a chair in the examining room, with her legs twisted around each other like a pretzel.

She had learned from experience that doing this delayed the onset of feeling lightheaded when she was upright. By working the muscles of the legs against each other, blood is squeezed upward in the body toward the heart. When there is deficient reflexive sympathetically-mediated vasoconstriction during orthostasis, pretzel legs help maintain venous return. Adopting the same posture is a



Twisting the legs around each other like a pretzel is a sign of orthostatic intolerance.

countermeasure in patients with autonomically mediated presyncope.

It is common for a patient with orthostatic intolerance to bring a bottle of water to the clinical encounter and sip from it periodically as the history is taken. I call this the “water bottle sign.” The patients often report that although drinking water continuously doesn’t eliminate the symptoms, not drinking water rapidly makes them worse.

To me this may be a clue as to the pathophysiology of chronic orthostatic intolerance. Perhaps the kidneys are less efficient in reabsorbing filtered water, and the water bottle sign is part of a behavioral compensation. The kidneys filter about 100 mL of plasma per minute. Since there are 1440 minutes in a day, this means the

kidneys filter about 144 liters per day. If normal urine output is 1.5 liters per day, this means that the kidneys are about 99% efficient in reabsorbing water. One might expect that even the slightest decrease in efficiency would result in a tendency to dehydration.

It happens that kidney cells possess water channels called aquaporins. A classmate of mine in medical school, Peter Agre, discovered aquaporins, and for this he received a Nobel Prize. It might be worth looking into whether there is a problem with aquaporins or with vasopressin, the anti-diuretic hormone that is the main water retention hormone of the body, in patients with chronic orthostatic intolerance and the water bottle sign.

A Bit of a Stretch

Joint hypermobility (“double jointedness”) seems to occur rather frequently among patients with postural tachycardia syndrome (POTS). When obtaining the medical history in a patient with chronic orthostatic intolerance, it is worthwhile to ask whether the patient is double jointed and if so to ask what sorts of things the patient can do that other people cannot.

Ehlers-Danlos syndrome (EDS) is an inherited connective tissue disease in which the patients have joint hypermobility, lax skin, a tendency to joint dislocation or subluxation, musculoskeletal pain, and



Two components of the 9-point Beighton score

easy bruising. EDS patients often have “Marfanoid” appearance, in that they are tall, thin, have long arms and legs, and have long thin fingers (arachnodactyly, or “spider fingers”).

POTS occurs frequently in EDS. One possible explanation for this association is the a problem with collagen in blood vessel walls makes them more stretchy or compliant, so that blood tends to pool in the abdomen or pelvis during prolonged standing.

The Beighton score is used to gauge the severity of joint hypermobility, based on 5 tests. The Beighton score is calculated as follows:

1. One point for each little finger that you can bend backwards by more than 90 degrees.

2. One point for each thumb that you can touch to your forearm when bent backwards.
3. One point for each elbow that you can bend backwards.
4. One point for each knee that you can bend backwards.
5. One point if while standing you can bend forward and place your palms on the ground with your legs straight.

PHYSIOLOGICAL TESTS

The Valsalva Maneuver

Despite its apparent simplicity, the Valsalva maneuver test is one of the most important clinical physiological tests for autonomic failure.

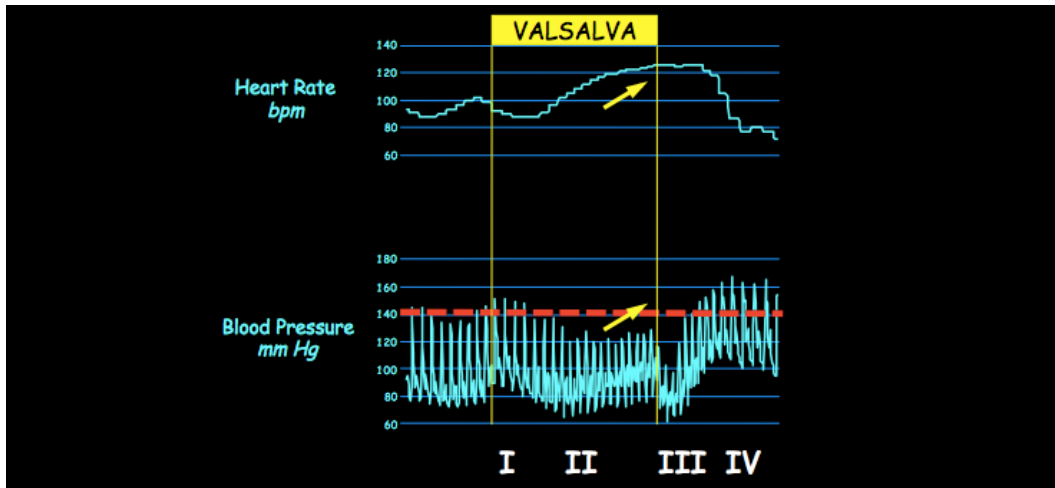
In the Valsalva maneuver, the patient blows against a resistance for several seconds and then relaxes.

The maneuver consists of blowing against a resistance for several seconds and then relaxing. Often the patient is asked to blow into a tube connected to a blood pressure gauge, moving the gauge's needle to a particular pressure (30-40 mmHg) and keeping the needle there for 10-15 seconds.

In Phase I, just after starting to squeeze, the blood is forced out of the chest, and the blood pressure increases briefly. This is mechanical and has nothing to do with reflexes.

As you continue to strain, the high pressure in the chest and abdomen results in less blood reaching the heart, and the heart pumps less blood, so normally in Phase II the blood pressure falls. Input to the brain from baroreceptors goes down.

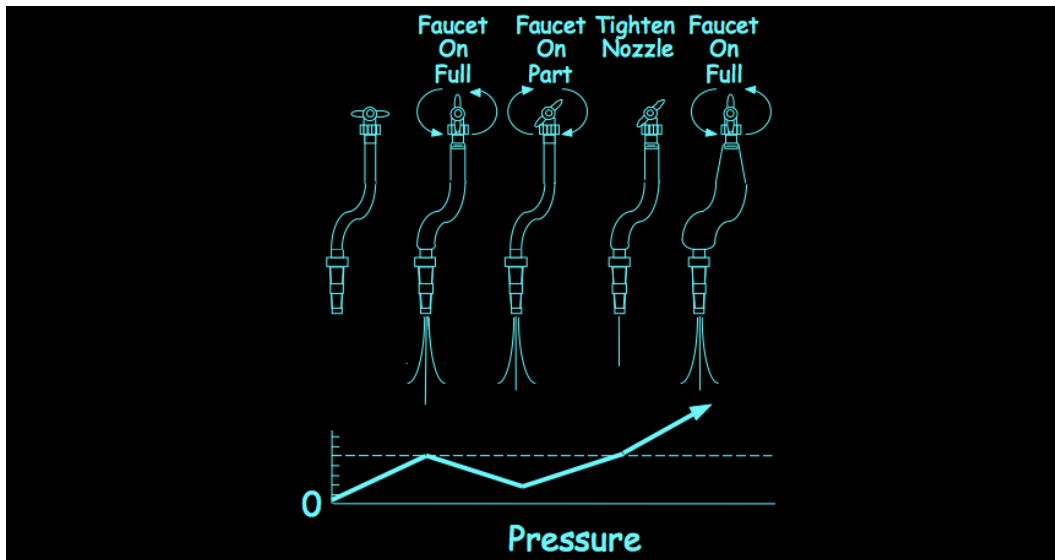
The brain picks up on this immediately and directs a reflex to occur in



Normal blood pressure (BP) and heart rate (HR) responses during the 4 phases of the Valsalva maneuver.

which outflows in the sympathetic noradrenergic system (NE) increase, norepinephrine, the chemical messenger of the SNS, is released, the norepinephrine binds to its receptors in the blood vessel walls, and the blood vessels tighten. At the end of Phase II blood pressure therefore increases, even though the heart is still pumping out less blood.

To understand this reflex better, think of the pressure in a garden hose. Turning down the faucet decreases the pressure in the hose, but you can bring the pressure back up by tightening the nozzle. The brain uses the sympathetic noradrenergic system to tighten the vascular nozzle, and so the blood pressure increases at the end of Phase II.

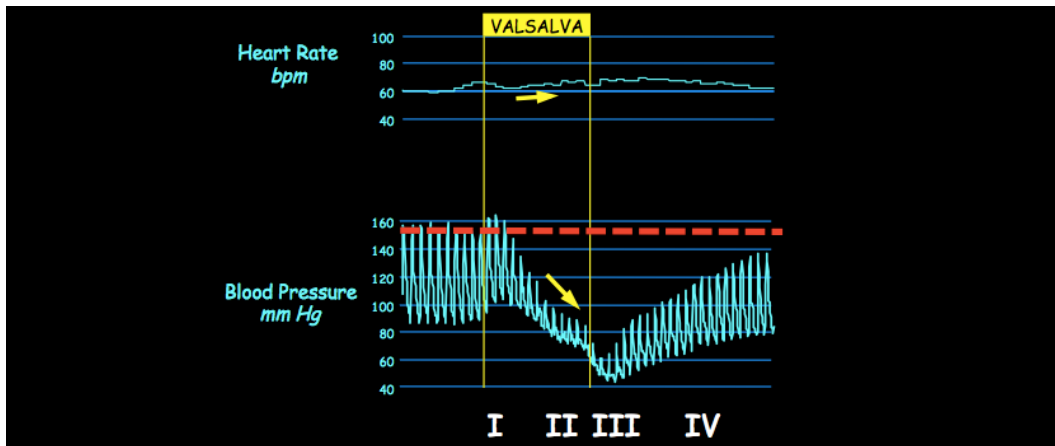


The garden hose analogy can help understand reflexive regulation of blood pressure associated with the Valsalva maneuver.

During Phase II the heart rate normally goes up, due to withdrawal of parasympathetic nervous system outflow to the heart via the vagus nerve.

Then you relax. Momentarily, in Phase III the blood pressure falls—a kind of mirror image to the increase in Phase I. The decrease in pressure in Phase III has nothing to do with reflexes.

Finally, in Phase IV the patient is relaxed, and there is no impediment



Abnormal blood pressure (BP) and heart rate (HR) responses to the Valsalva maneuver, indicating failure to regulate the sympathetic and parasympathetic nervous systems correctly.

in blood getting to the heart. The heart pumps the blood, but it pumps the blood into the reflexively constricted vasculature, and so the blood pressure overshoots the baseline value. It's as if you turned the faucet back up to where it was originally, but you forgot to loosen the nozzle.

Because of the overshoot in pressure, the heart rate rapidly reflexively falls back to baseline.

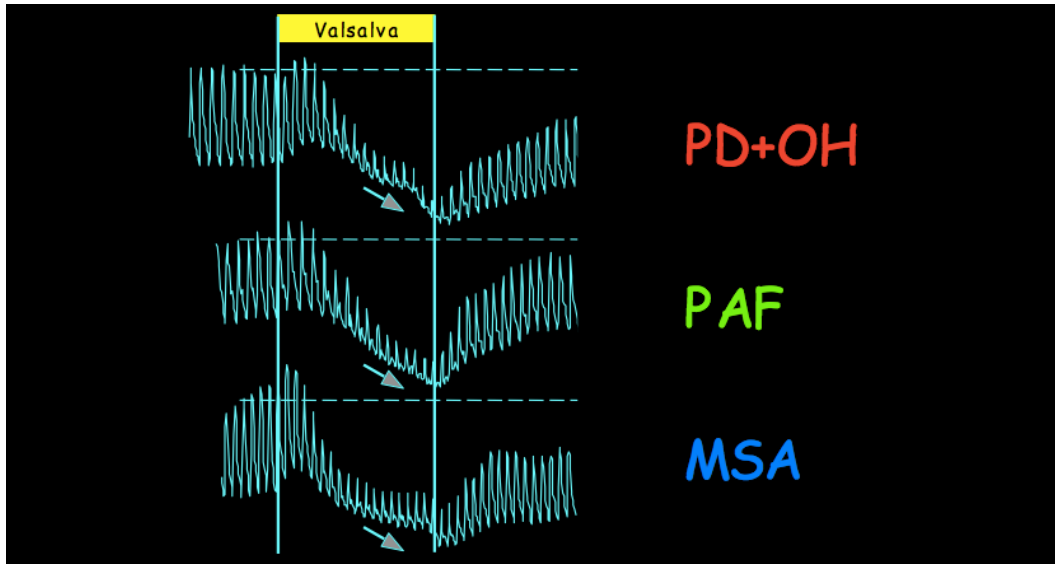
In a patient with failure of this reflex, whether because there is a decrease in afferent information from the baroreceptors to the brain, or because the brain doesn't act on that information due to a brain disease, or because the sympathetic nerves are gone, or because norepinephrine isn't released, or because the adrenoceptors receptors

are blocked, you get the same abnormal pattern of blood pressure during and after the Valsalva maneuver. In Phase II the blood pressure goes down progressively, because the patient can't tighten the vascular nozzle, and in Phase IV the pressure returns slowly to the baseline value but doesn't overshoot, for the same reason.

In most (but not all) forms of chronic autonomic failure manifesting as orthostatic hypotension, the heart rate doesn't change as much as it should given the larger fall in pressure. The extent of increase in heart rate (or more formally the extent of decrease in the interbeat interval) per mmHg decrease in systolic blood pressure during Phase II is a measure of baroreflex-cardiovagal gain.

Note that one must monitor the blood pressure changes beat-to-beat in order to diagnose sympathetic neurocirculatory failure based on the Valsalva maneuver. Until recently, such monitoring required insertion of a catheter into an artery. Since neurologists rarely feel comfortable doing this, they usually settle for recording only the peak and trough pulse rates during and after performance of the maneuver. This may enable a diagnosis of parasympathetic neurocirculatory failure but cannot diagnose sympathetic neurocirculatory failure. Nowadays there are non-invasive devices available to track blood pressure beat-to-beat and detect baroreflex-sympathoneural failure.

It is important to bear in mind that the finding of abnormal blood



The same abnormal pattern of beat-to-beat blood pressure occurs in different autonomic failure syndromes.

pressure responses to the Valsalva maneuver is valuable for diagnosing sympathetic neurocirculatory failure but is of no value in differential diagnosis among autonomic failure syndromes. For instance, the same abnormal pattern occurs in Parkinson disease with orthostatic hypotension, the Parkinsonian form of multiple system atrophy, and pure autonomic failure.

Tilt Table Testing

Tilt table testing is done to see if standing up (orthostasis) provokes a gradual, progressive fall in blood pressure (orthostatic hypotension), a

period of blood pressure instability followed by a sudden fall in blood pressure (neurally mediated hypotension), an excessive increase in pulse rate, as in postural tachycardia syndrome (POTS), or fainting (also known as autonomically mediated syncope, neurally mediated syncope, neurocardiogenic syncope, or reflex syncope).

The testing itself is simple. The patient lies on a stretcher-like table, straps like seat belts are attached around the abdomen and legs, and the patient is tilted upright at an angle. The exact angle used varies from center to center and may be from 60 degrees to 90 degrees. The tilting goes on for up to many minutes (this again varies from center to center).

If the patient tolerates the tilting for this period, then the patient may receive a drug, such as isoproterenol or nitroglycerine, which may provoke a sudden fall in blood pressure or loss of consciousness. (Most autonomic centers no longer do this, because of the possibility of false positive test results.)

As soon as the test becomes positive, such as by a sudden fall in blood pressure, the patient is put back into a position lying flat or with the head down. Sometimes fluid is given by vein. Consciousness rapidly returns once the patient is put back down; however, symptoms such as a sense of imbalance, disorientation, or headache can continue for hours or even days later.

Tilt table testing is a form of provocative test. The doctors are hoping



Tilt table testing usually is done with a motorized tilt table.

to reproduce the patient's problem in a controlled laboratory situation.

Tilt table testing is used to evaluate patients with a complaint of fainting or inability to tolerate prolonged standing.

The testing is quite safe when done by experienced personnel, in a setting where emergency backup is available.

There are some disadvantages of tilt table testing. One is false-positive test results, especially when a drug is used. In a false-positive test, the results of the test are positive, but some healthy people can have a positive test result, so that a positive test result might not actually mean that anything really is “wrong.”

More importantly, a false-positive result would lead the doctor to conclude that the condition is fainting, a relatively benign situation, whereas the patient actually has a serious medical problem. This is

what happened in the case of the basketball star Reggie Lewis, as discussed below, and in the ironic case of one of his cardiologists, Dr. Thomas Graboys.

Tilt table testing also might not reproduce the patient's problem—a false-negative test result.

Another disadvantage is that most tilt table testing does not provide information about disease mechanisms. This means that, beyond verifying the patient's complaints, the testing does little to suggest treatments that might be effective.

“Augmented” tilt table testing involves measurements of physiological functions such as forearm vascular resistance and sampling blood for assays of levels of norepinephrine and adrenaline. Augmented testing can provide information about mechanisms; however, few centers offer this form of tilt table testing.

Standard tilt table testing is not useful in patients with a persistent fall in blood pressure each time they stand up (orthostatic hypotension), because the results are a foregone conclusion: the blood pressure will fall progressively during the tilting. Augmented tilt table testing, however, can help determine if the orthostatic hypotension results from a form of sympathetic nervous system failure.

The Reggie Lewis Case

Reggie Lewis was a star basketball player for the Boston Celtics. In game 1 of the 1993 Eastern Conference First Round of the NBA Playoffs, on April 29, 1993, he collapsed on the floor. He came back later and finished with 17 points. He was evaluated by a 12-member cardiology “dream team” that included Dr. Thomas Graboys at the New England Baptist Hospital. They thought he had a form of cardiomyopathy and recommended that Lewis cease playing.

Needless to say, millions of dollars were at stake. Lewis went for a second opinion, which was provided by Dr. Gilbert Mudge of the Peter Bent Brigham Hospital. Mudge opined that Lewis had “athlete’s heart” and neurocardiogenic syncope—benign conditions—and could resume playing. This assessment became one of the most widely publicized and second-guessed opinions in the annals of medicine. According to a New York Times article, a key procedure that led to Mudge’s opinion was a tilt table test. During head-up tilting at 60 degrees from horizontal, Lewis reported the same lightheadedness that he had experienced before collapsing on the Celtics’ NBA court.

The tilt table test yielded false-positive results. Before Lewis ever played another NBA game, while shooting hoops at Brandeis University on July 27, 1993 he collapsed again—and died. He was autopsied and found to have an abnormal, enlarged, extensively scarred heart, but the exact cause of death was never clarified. His death was attributed variously to hypertrophic

cardiomyopathy, a viral myocarditis, or even cocaine cardiotoxicity. A lawsuit filed by the widow against Mudge resulted in a mistrial.

The important thing is that he didn't have mere fainting. He had a false-positive tilt table test.

The story of the Reggie Lewis case leads ironically to the story of one of his cardiologists, Dr. Thomas Graboys, discussed in the section on dementia with Lewy bodies.

Sweat Tests

Sweating is an important way people regulate body temperature in response to external heat. The brain increases sweating by directing an increase in sympathetic cholinergic system (SCS) traffic to sweat glands in the skin. The chemical messenger, acetylcholine, is released, the acetylcholine occupies muscarinic receptors on the sweat glands, and the glands secrete sweat.

Sweat tests evaluate a particular part of “automatic” nervous system function.

One can examine SCS function from the sweating response to external heat (thermoregulatory sweat test, TST). Sweat production can be visualized by sprinkling starch with iodine or other indicator powder (e.g., alizarin red) all over the body. When the powder is wetted, the

powder turns color. One can then photograph the body and see which parts sweated. This sort of sweat testing can be informative in detecting small fiber neuropathy, sympathetic cholinergic denervation in the feet or hands, or denervation in large areas of the trunk.

When the skin becomes sweaty, the ability to conduct electricity increases because of the salt and water in the sweat, and one can monitor the electrical conductivity. Sweat increases local humidity, and one can also monitor the humidity in a chamber strapped to a limb and applied to the skin. One can also take pictures of sweat droplets or obtain a latex impression of the droplets.

The galvanic skin response (GSR), or skin sympathetic test (SST), is part of polygraphic “lie detector” testing. When a person is suddenly distressed, or a small electric shock is delivered, increased SCS (and possibly sympathetic adrenergic system) activity evokes sweating.

Advantages of sweat tests are that they are generally safe, simple, and quick. A disadvantage is that they mainly or only measure physiological changes as a result of release of acetylcholine from sympathetic nerves. There are some dysautonomias (such as Parkinson disease with orthostatic hypotension) where the patient has normal sweating.

Another disadvantage is that sweat tests are only indirectly and complexly related to activity of the sympathetic noradrenergic system, and in most cases they provide little information about the mechanism

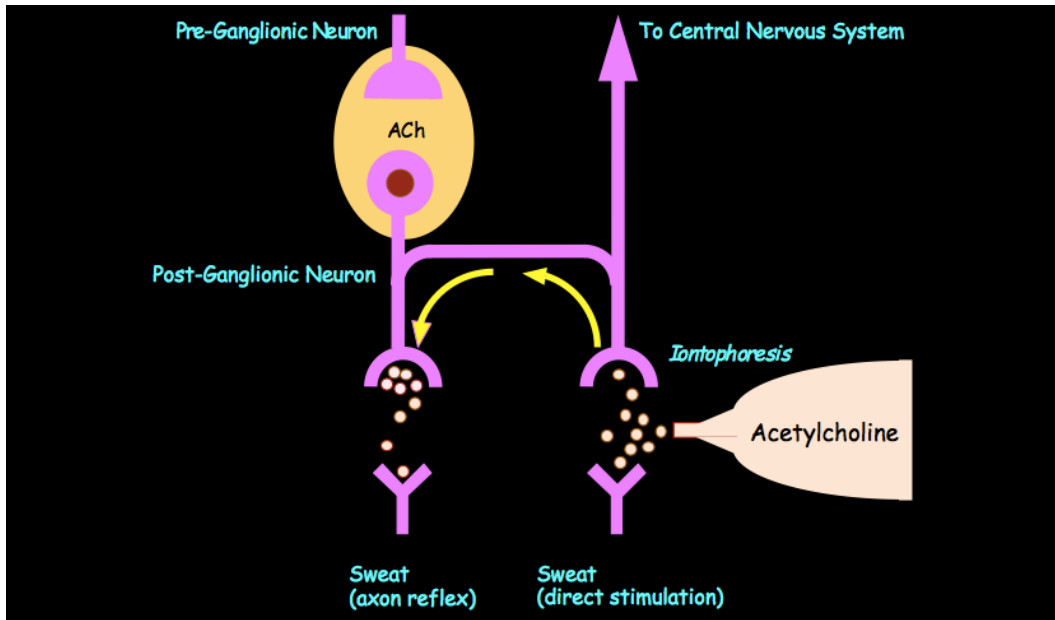
of the dysautonomia. Drugs that block receptors for acetylcholine are used commonly for urinary problems, and these drugs can interfere with results of the test.

The QSART

“QSART” stands for “Quantitative Sudomotor Axon Reflex Test.”

This test is a form of sweat test. Sweating in response to altered environmental temperature results from the effects of the chemical messenger, acetylcholine, released from sympathetic nerve terminals near sweat glands in the skin. This arrangement is different from that for alterations in the pulse rate and blood pressure that result from effects of norepinephrine released from sympathetic noradrenergic nerves in the heart and blood vessel walls. The QSART is a test of the ability of sympathetic nerves in the skin to release acetylcholine and increase sweat production.

In the QSART procedure, dried air is pumped at a controlled rate through a small plastic capsule placed on the skin. When the person sweats, the humidity in the chamber increases. This provides a measure of sweat production.



The QSART is a special form of sweat test.

For QSART testing, acetylcholine is applied to a nearby patch of skin, by a special procedure called iontophoresis. The locally applied acetylcholine evokes sweating at the site where it is given, but in addition, by way of a type of reflex called an axon reflex, sympathetic nerves under the nearby plastic capsule release the body's own acetylcholine. This results in sweat production measured by increased humidity in the capsule.

If a person had a local loss of sympathetic cholinergic nerves, then applying acetylcholine to the patch of skin near the test capsule would not lead to increased sweating or increased humidity in the test capsule, although the acetylcholine would increase sweating directly

where it was applied. If the person had a brain disease that prevented increases in sympathetic cholinergic nerve traffic during exposure to increased environmental temperature, then the person would not be able to increase the humidity in the capsule in response to an increase in the room temperature, and yet the person would have a normal QSART response.

By this sort of neuropharmacologic test, doctors can distinguish sympathetic cholinergic system failure due to loss of sympathetic cholinergic nerves from failure due to abnormal regulation of nerve traffic in intact nerves.

Advantages of the QSART are that it is generally safe, quick, quantitative, and easy to perform. There are also several disadvantages. The equipment required is expensive. As in other tests where the key factor being measured is physiological (in this case, sweat production), the results are indirect. For instance, if the patient had a problem with the ability to make acetylcholine in the nerve terminals, with the ability of acetylcholine to bind to its receptors in the sweat glands, or decreased numbers of sweat glands, the patient would have the same abnormal QSART responses as if the sympathetic cholinergic nerves were lost. QSART results may or may not identify problems in regulation of the heart and blood vessels by other parts of the autonomic nervous system. In other words, the QSART results might not be representative.

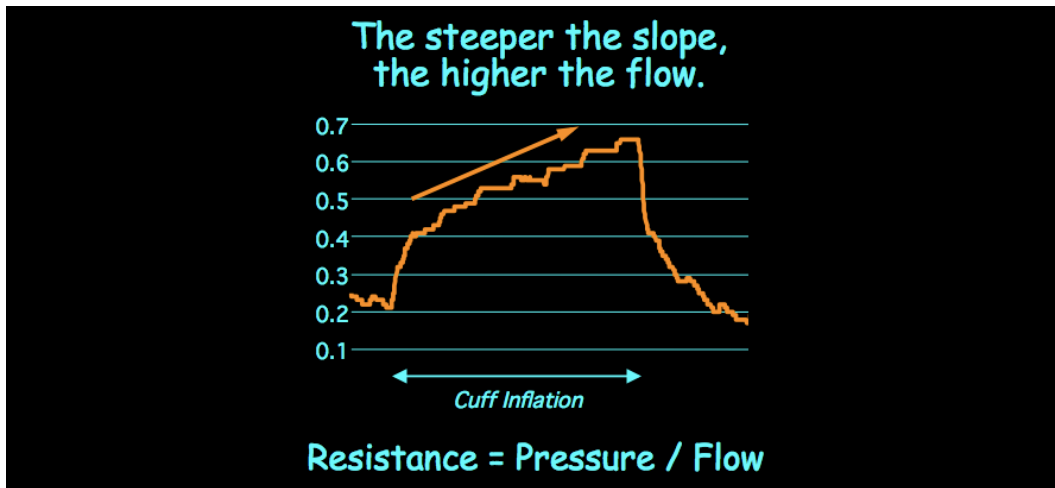
QSART testing is a useful way to detect loss of nerve fibers in the feet, as occurs in small fiber neuropathies and “neuropathic” POTS.

Forearm Blood Flow

Blood flow in the forearm can be measured non-invasively using an automated blood pressure cuff and a bracelet-like device.

Measuring forearm blood flow is useful to test whether the patient tightens blood vessels reflexively, as normally happens during assumption of upright posture.

One non-invasive way to measure forearm blood flow is by a technique called impedance plethysmography. A blood pressure cuff is attached around the upper arm, and a special bracelet-like device called a strain gauge is attached around the upper forearm. The strain gauge measures stretch very sensitively. For a measurement of forearm blood flow, the blood pressure cuff is inflated rapidly using a special cuff inflator to just above the venous pressure but below the diastolic blood pressure (typically at 40 mmHg). This is like tightening a tourniquet around the upper arm to obtain a blood sample. Because the cuff pressure is above the venous pressure, blood in the forearm and hand can't get past the cuff, and because the cuff pressure is below the arterial pressure, blood can still enter the forearm and



One way to measure forearm blood flow is a method called impedance plethysmography.

hand. In this situation, the volume of the forearm expands slightly, and the strain gauge senses the increase in volume.

If the rate of blood flow into the forearm were high, then the volume of the forearm would increase rapidly after the cuff was inflated; and if the rate of blood flow were low, then the volume of the forearm would increase more slowly. By a simple calculation you can estimate the blood flow into the forearm, from the rate of increase in the volume of the forearm after the cuff is inflated. Usually, measurement of forearm blood flow is done at least five times, to obtain a reliable average value.

Once the rate of forearm blood flow (FBF) is known, the forearm

vascular resistance (FVR) is calculated from the average blood pressure (mean arterial pressure, MAP) divided by the forearm blood flow. This is a similar calculation as for measuring total peripheral resistance (TPR) from the mean arterial pressure (MAP) divided by the cardiac output (CO). In the garden hose analogy referred in the presentation of the Valsalva maneuver, the FVR would correspond to the extent of tightening of the nozzle.

When you stand up, the forearm vascular resistance normally increases. This is because of reflexive activation of the sympathetic noradrenergic system. When a person stands up or is tilted on a tilt table as part of tilt-table testing, the amount of blood ejected by the heart per minute falls, due to the force of gravity, which tends to pool blood in the legs and lower abdomen and decreases venous return to the heart. The brain directs an increase in sympathetic noradrenergic system outflows, norepinephrine is released from nerve terminals in blood vessel walls in the forearm and hand, and the forearm vascular resistance (FVR) goes up. In sympathetic neurocirculatory failure, the FVR doesn't increase like it should and may not increase at all. In fainting, the FVR typically decreases, due to adrenaline-induced relaxation of blood vessels in skeletal muscle. In patients with low blood volume, FVR may be high even during supine rest, as part of a compensation to maintain blood pressure.

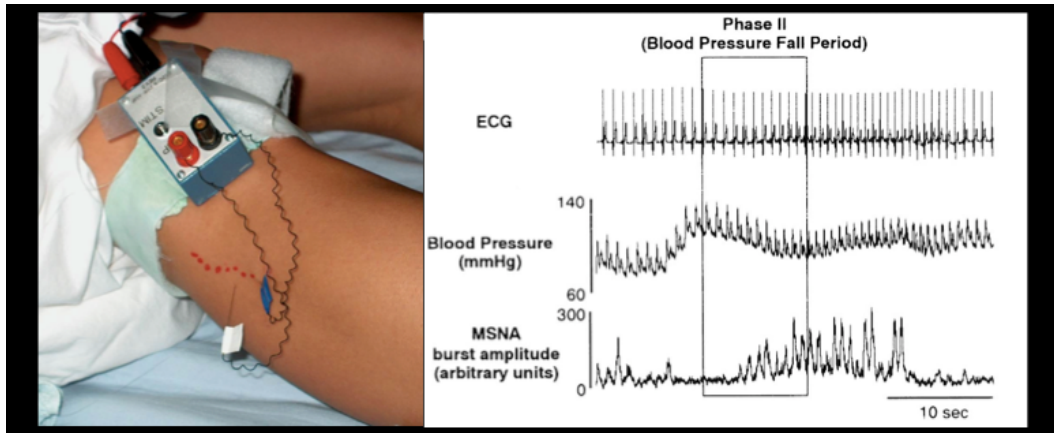
Sympathetic Microneurography

One can monitor sympathetic outflow to skeletal muscle via a needle electrode inserted into the peroneal nerve, which is in the “funny bone” outside and just below the knee. Sometimes the measurement is abbreviated MSNA, for muscle sympathetic nerve activity.

Bursts of MSNA are related to baroreflex function. The bursts are tied to the heartbeat and are called “pulse-synchronous.” When the blood pressure decreases, MSNA increases reflexively, due to activation of sympathetic noradrenergic system outflow to the skeletal muscles. The frequency of pulse-synchronous bursts goes up.

An advantage of monitoring MSNA is the ability to track SNS responses to a variety of stimuli rapidly and in real time. Carrying out MSNA measurements requires substantial technical training and experience.

Identifying nerve traffic as MSNA often requires assessing effects of baroreflex activation or inhibition on the signal, such as by breath holding or performing a Valsalva maneuver. In patients with chronic autonomic failure, MSNA can be difficult to measure because of the lack baroreflex-mediated, pulse-synchronous bursts of nerve traffic.



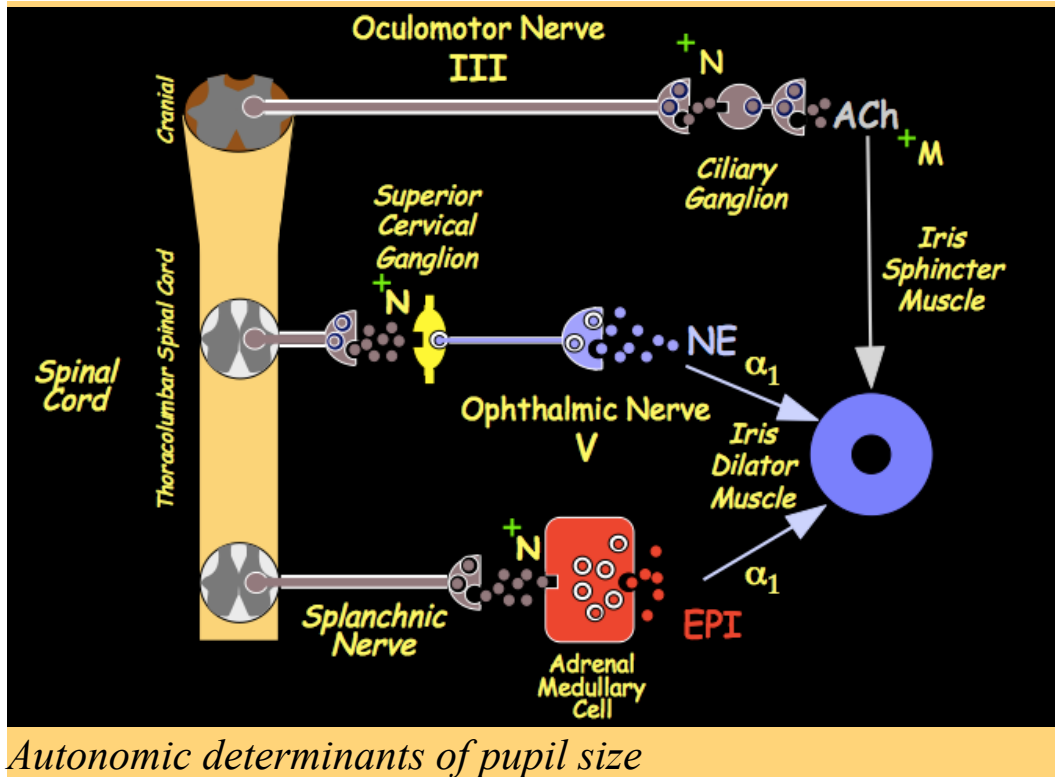
During the Valsalva maneuver, the decrease in blood pressure in Phase II is associated with a reflexive increase in bursts of skeletal muscle sympathetic nerve traffic.

Sympathetic noradrenergic outflow to the skin is not so sensitive to baroreflexes and is more sensitive to psychological phenomenon such as startle.

Pupillometry

The pupils of the eyes receive both parasympathetic nervous system (PNS) and sympathetic noradrenergic system (SNS) innervation.

The PNS innervation of the pupils is derived from the Edinger-Westphal nucleus, located in the midbrain of the brainstem. The nerve fibers synapse in the ciliary ganglion and travel with the oculomotor



nerve, which is the third cranial nerve.

The SNS innervation of the pupils is derived from pre-ganglionic neurons in the thoracic spinal cord. The nerve fibers synapse in the superior cervical ganglion in the neck and travel with the ophthalmic nerve, which is a branch of the trigeminal nerve, the fifth cranial nerve.

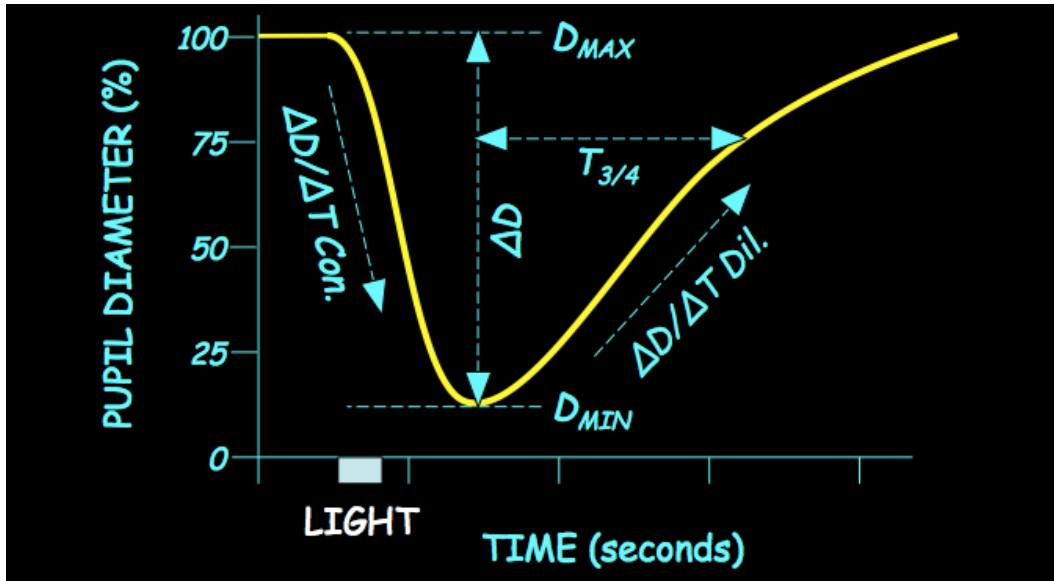
Pupil constriction evoked by PNS stimulation is mediated by acetylcholine acting at muscarinic receptors on iris sphincter muscle

cells. The sphincter muscle cells are arranged circularly in the iris, and so when they contract the pupil gets smaller. The pupil dilation evoked by SNS stimulation is mediated by norepinephrine acting at alpha-1 adrenoceptors on iris dilator muscle cells. The iris dilator cells are arranged radially (like spokes on a bicycle wheel) in the iris, and so when they contract the pupil gets larger.

Activation of the sympathetic adrenergic system (SAS), such as during distress, causes release of adrenaline into the bloodstream. Adrenaline also acts at the alpha-1 adrenoceptors on iris dilator muscle cells and dilates the pupils. SAS activation probably explains the pupil dilation that typically occurs when people faint.

Pupillometry involves tracking the dynamics of pupil size in response to a brief light stimulus. This is a simple, non-invasive autonomic function test. In response to a brief light stimulus, the pupils constrict, due to a rapid increase in PNS activity. After the light stimulus, the pupils slowly re-dilate. The rate of re-dilation seems to involve a contribution of the SNS, since patients with Horner's syndrome (discussed below) not only have a small pupil but also have been reported to have a delay in the return of pupil diameter toward baseline (prolonged $T_{3/4}$ in the diagram).

The pupillary light reflex is too rapid to involve adrenaline.



Pupil diameter responses to a brief light stimulus

How pupillometry results relate to abnormalities of particular components of the autonomic nervous system is a matter of current research.

Horner's Syndrome

Horner's syndrome (also called Horner-Bernard and Bernard-Horner syndrome, depending on your loyalty to Claude Bernard) involves the triad of ptosis (lid lag), miosis (constricted pupils), and anhidrosis (lack of sweating) on the affected side of the face.

Horner's syndrome usually reflects loss of input from the sympathetic



Horner's syndrome. There is a droopy left eyelid (ptosis), a smaller pupil (miosis), and decreased sweating.

noradrenergic system (SNS) and sympathetic cholinergic system (SCS), while PNS effects on the pupils are unopposed.

Sympathetic nerves to the face course from the thoracic spinal cord through ganglia before ascending in the chest and neck to the head. A tumor in the chest or neck that involves the sympathetic chain can manifest clinically as Horner's syndrome.

A Famous Photo



These drawings are the same, except for two aspects. What's different about the eyes on the left compared to the right?

One of the most famous and haunting photographs ever published appeared on the cover of National Geographic in June, 1985. Taken by Steve McCurry, it was entitled, “Afghan Girl.” Why is the photo so powerful?

In the drawings above, the eyes are identical except for only two aspects. First, the pupils of the eyes on the left are severely constricted. Second, the eyelids of the eyes on the left are raised. This is what happens when a person is startled. The combination of miosis and raised eyelids produces a penetrating, glaring stare that is scary. The person seems deranged and unpredictable, like the Joker in the Batman series.

An alternative explanation for miosis alone could be an opiate. Opiates such as morphine and heroin produces miosis, probably via stimulation of neurons in the Edinger-Westphal nucleus. Afghanistan has been the world's most prolific producer of illicit opiates, and the girl in the photo was living in a refugee camp in Pakistan during the war in Afghanistan against the Soviet occupation. An opiate,

however, would not explain the enlarged whites of her eyes.

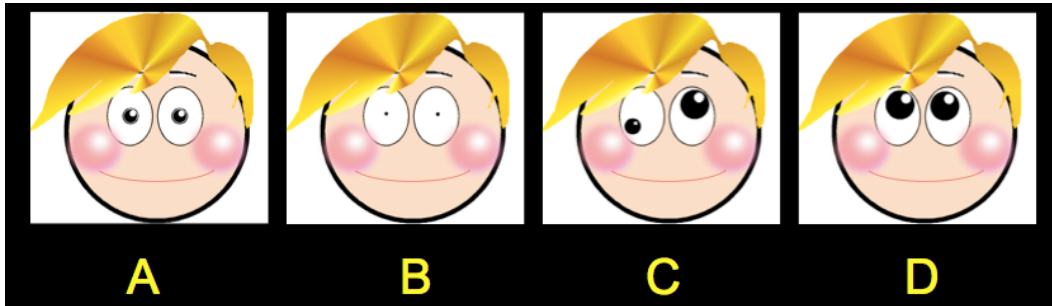
In an interview many years later the photographer recalled that the girl was in school at the time. At first she covered her face with her hands, but her teacher told her to take her hands down and let him take her picture because of the global importance of their story. When the shutter was released, the girl had just uncovered her face. In other words, the pupillary light reflex could have contributed to the miosis. To me, though, there had to be an element of startle to explain the enlarged whites of the girl's eyes.

Students of Pupils

Cartoonists frequently exploit effects of alterations in autonomic nervous system outflows to the pupils to convey the psychological state of the characters.

Look at the four faces. One of them is neutral, one is cute and sweet, one is disoriented, and one is startled. Which is which?

Cartoonists (and probably you) appreciate that pupil constriction instinctively conveys startle, inequality of the pupils conveys disorientation, and enlarged pupils convey cuteness (as in “belladonna,” the drug used for centuries to enhance attractiveness by enlarging the pupils). A is neutral, B is startled, C is disoriented, and D is cute and sweet.



Cartoonists have long exploited different appearances of the pupils to convey different neurobehavioral states.

When I've asked doctors the same question, they often get it wrong, because of what they are taught about the "stress response." Based on Cannon's fight-or-flight response and the emergency function of the "sympathoadrenal system," distress raises adrenaline levels and increases sympathetic nervous system outflows, and these changes would produce dilation of the pupils (mydriasis). By this rationale doctors can go against their own intuition and guess that D corresponds to startle. Mydriasis attending distress takes time, however. Recall that the sympathetic post-ganglionic nerves are non-myelinated and conduct signals relatively slowly. The immediate startle response involves miosis, probably because of rapid effects of parasympathetic nervous system stimulation. The parasympathetic innervation to the eyes comes from the brainstem and doesn't involve slow-conducting non-myelinated fibers.

The vagus nerves, which convey parasympathetic nervous system

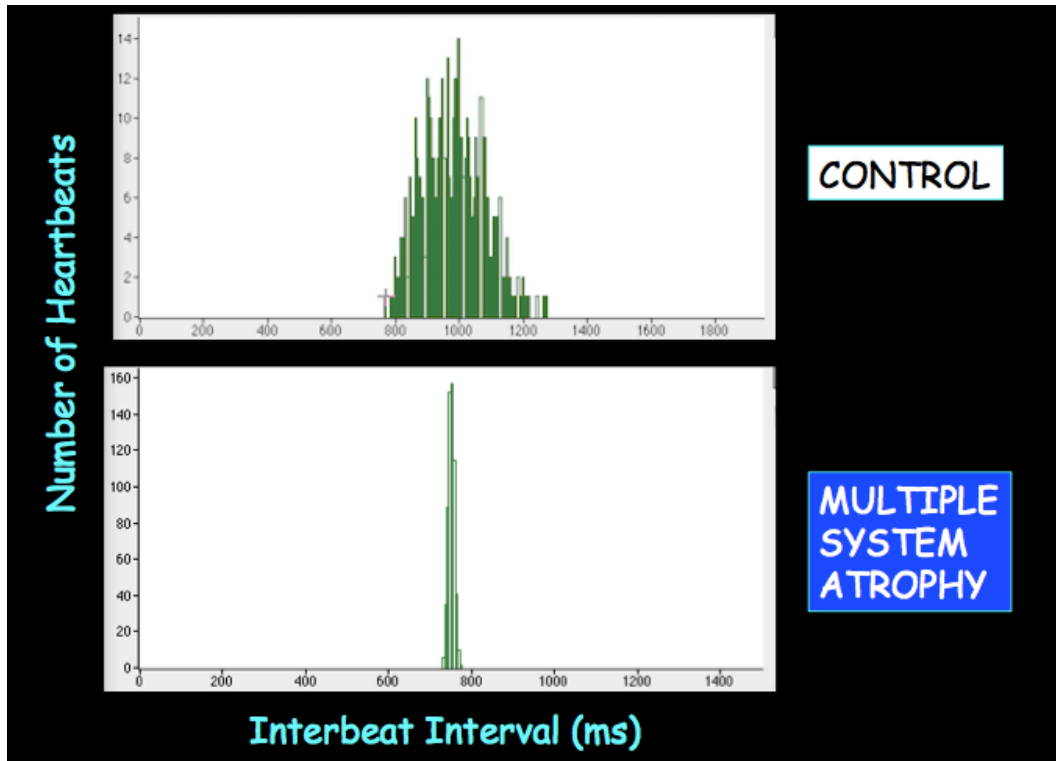
signals to the heart from each side of the lower brainstem, are also myelinated and conduct signals rapidly. Sudden vagal stimulation during startle would therefore be expected cause a rapid drop in heart rate or a brief period of slow conduction of electrical signals within the heart. This is why when you are startled you feel that your heart has “skipped a beat.” Startle precedes distress, and the bradycardia or heart block caused by vagal activation precedes the fast and pounding heartbeat resulting from sympathetic noradrenergic and adrenergic system stimulation.

Doctors are also taught, in this case correctly, that a “blown,” dilated pupil on one side and deviation of the eye on that side can disclose a catastrophe inside the head, such as from a clot on the brain. I think cartoonists exploit the instinctive communication value of this appearance to convey disorientation, as in panel C.

Heart Rate Variability

The Sign of a Healthy Heart

When you take in a slow, deep breath, your pulse rate normally increases, and when you then breathe out, your pulse rate falls. The wave-like rhythmic change in the heart rate due to breathing is called respiratory sinus arrhythmia. Despite the word, arrhythmia, meaning “lacking rhythm,” respiratory sinus arrhythmia is quite rhythmic and



Heart rate variability in the time domain. Chronic autonomic failure syndromes such as multiple system atrophy involve low heart rate variability.

quite normal. These changes result mainly from modulation of vagus nerve traffic to the heart. The famous Dutch cardiologist, Karel Frederik Wenckebach, wrote in the early 1900s that a variable pulse rate is the sign of a healthy heart.

If you recorded the cardiac interbeat interval across many heartbeats and graphed the number of beats in bins of interbeat intervals, you

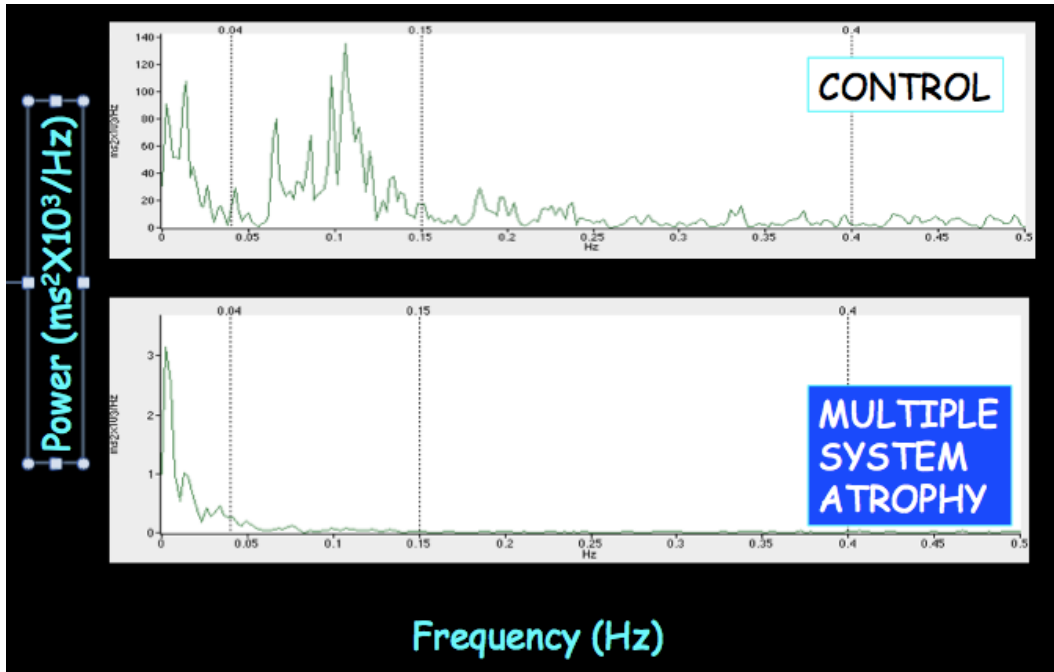
would see a bell-shaped curve. The more variable the heart rate, the wider the bell-shaped curve. This is called analysis of heart rate variability in the time domain.

With aging, many forms of heart disease such as congestive heart failure, and in most forms of chronic autonomic failure, the heart rate becomes more stable. The bell-shaped curve becomes narrower. This is probably not from altered autonomic outflows to the heart but from decreased reflexive modulation of those outflows.

Power Spectral Analysis of Heart Rate Variability

This test is much simpler than the fancy name suggests. Normally, a person's heart rate is not constant. The pulse rate increases when the person breathes in and then decreases when the person breathes out.

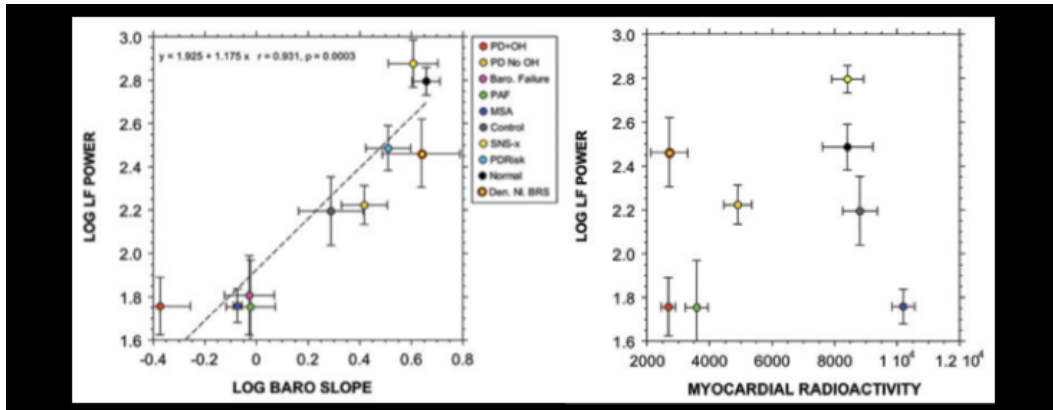
This means that the pulse rate normally oscillates in a wave-like pattern. Another form of analysis of heart rate variability is in the frequency domain. If one graphed the size of the oscillation as a function of the frequency of the heartbeats, then at the frequency of breathing, which is about once every 8 seconds, corresponding to $8/60$ or 0.13 cycles per second (hertz), there would a peak of "power." In people who have failure of the parasympathetic nervous system, there is little or no respiratory sinus arrhythmia, and there is no peak of power at the frequency of breathing.



Heart rate variability in the frequency domain. At all frequencies there is low power in multiple system atrophy.

This sort of analysis typically reveals a second peak of power, at a lower frequency than the frequency of breathing. Researchers have thought that low frequency power of heart rate variability is related to sympathetic nervous system influences on the heart; however, the parasympathetic nervous system also affects low frequency power.

Other researchers have disagreed with the notion that power spectral analysis of heart rate variability can assess sympathetic innervation of the heart and have proposed instead that low frequency power is more



Across a variety of dysautonomias the low frequency power is related to baroreflex function but not to sympathetic innervation of the heart.

a measure of the ability to modulate autonomic outflows to the heart by way of the baroreflex. Strong support for this view comes from the fact that across a variety of dysautonomias the log of low frequency power is related to the log of baroreflex-cardiovagal gain.

Power spectral analysis of heart rate variability offers the advantages of being safe, technically easy, and fast.

The main disadvantage is that the meanings of low frequency power (and of the low:high frequency ratio, proposed to reflect “sympathovagal balance”) remain unsettled.

Ambulatory Blood Pressure Monitoring

Ambulatory blood pressure monitoring, or ABPM, refers to automatic recording of blood pressure at pre-set time intervals during activities of daily life.

Ambulatory blood pressure monitoring can be used to detect high blood pressure at night (nocturnal hypertension).

ABPM can be valuable to assess whether the patient has the normal “dipping” of blood pressure that occurs during the night. Non-dipping often occurs in patients with neurogenic orthostatic hypotension, because during the day the patients have relatively low blood pressure when they are upright, and at night they have relatively high blood pressure when they are lying down (nocturnal hypertension).

ABPM is quite useful to assess variability of blood pressure. Patients with arterial baroreflex failure typically have large swings of blood pressure during the day and night.

Some patients have “white coat hypertension,” meaning their blood pressures are high in the doctor’s office but are normal at home. ABPM can help diagnose white coat hypertension.

The Cold Pressor Test

In the cold pressor test, blood pressure is monitored when the patient

dunks a hand into a container of ice-cold water and keeps the hand immersed. This rapidly increases the blood pressure by increasing activity of the sympathetic noradrenergic system. In a patient with baroreflex failure and an intact sympathetic noradrenergic system, the cold pressor test would be expected to evoke an exaggerated increase in blood pressure, while in a patient with baroreflex failure and sympathetic noradrenergic denervation the pressor response would be blunted.

The cold pressor test can only be done for a minute or two. The stimulus is complex and dynamic because of the rapid development of pain, numbness, and distress. Patients with dysautonomia associated with burning pain in the skin (erythromelalgia) can have a remarkable ability to tolerate prolonged cold pressor testing.

Composite Autonomic Severity Scale

Autonomic laboratory checklists have been developed with the goal of aiding the diagnostic workup. Findings are weighted in terms of orthostatic intolerance, sexual failure, bladder disorder, diarrhea, gastroparesis, secretomotor disorder, constipation, vasomotor disorder, and pupillomotor disorder. A 10-point composite autonomic severity scale (CASS) allots 4 points for “adrenergic failure” and 3 points each for “sudomotor failure” and “cardiovagal” failure. Patients with a CASS score of 3 or less have no or mild autonomic failure, 4-6 moderate autonomic failure, and 7-10 severe autonomic failure.

This kind of lumped approach to autonomic failure may be worthwhile for clinical research purposes but can be unhelpful in individual diagnosis. The CASS is insensitive to failure of single components of the autonomic nervous system, such as in dopamine-beta-hydroxylase (DBH) deficiency. The prevalence in the relevant population is not taken into account—constipation in the elderly is common, while pupillometer dysfunction is uncommon. Within a particular domain, subtle differences can be crucial for differential diagnosis—urinary bladder dysfunction is found in both Parkinson disease with orthostatic hypotension (PD+OH) and in the parkinsonian form of multiple system atrophy (MSA-P), but urinary retention requiring self-catheterization is common in MSA-P and rarely if ever found in PD+OH. The term, “adrenergic failure,” is misleading, since no measure of adrenergic (as opposed to noradrenergic) failure is included. The composite scale also depends importantly on the particular center. Neurochemical and neuroimaging tests that are more sensitive and informative than physiological tests are not included.

DRUG TESTS

Tyramine

In the tyramine (TYR) infusion test, the drug tyramine is infused i.v. TYR taken up into the sympathetic nerves displaces norepinephrine

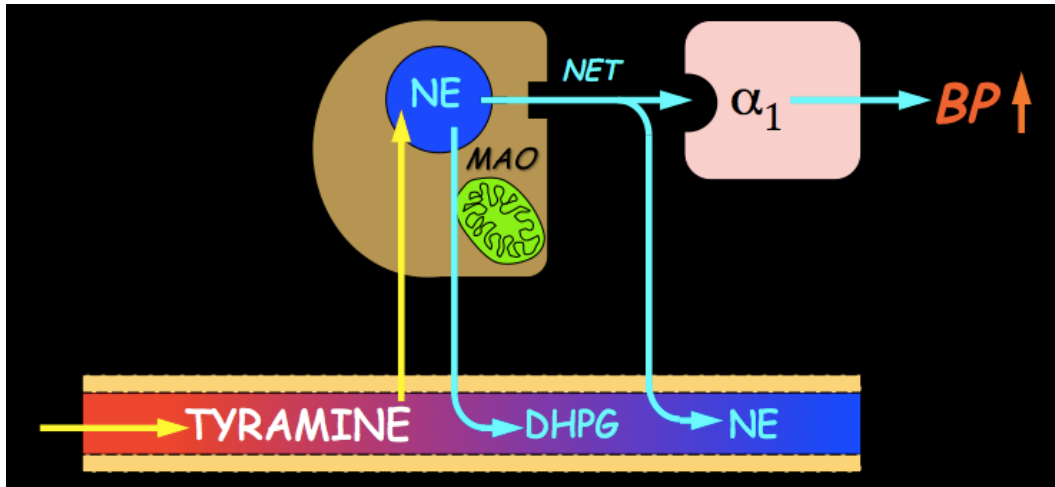


Diagram of the tyramine (TYR) infusion test.

(NE) from the vesicles. Some of the NE reaches its receptors on vascular smooth muscle cells, and the blood pressure goes up. Plasma NE and its breakdown product DHPG may also be measured.

Tyramine is a chemical that is found in some dietary constituents, such as hard cheese and red wine. Normally tyramine that is ingested is broken down in the gut and produces no effects on the body; however, if you are on a drug that interferes with this breakdown, then the tyramine can enter the bloodstream.

If a patient had autonomic failure due to a loss of sympathetic nerves, tyramine would not release norepinephrine from the nerves, because there would be no norepinephrine to displace. In such a patient tyramine would not increase the blood pressure by as much as if the

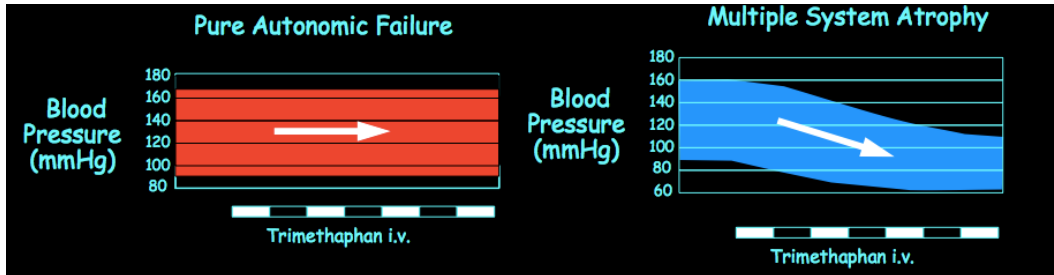
patient had an intact sympathetic noradrenergic system. In addition, such a patient would have relatively small increases in levels of norepinephrine and related compounds, such as dihydroxyphenylglycol (DHPG), in the plasma.

The enzyme that breaks down tyramine in the gut is called monoamine oxidase (MAO). MAO inhibitors are used to treat some psychiatric or neurological disorders. If a patient on an MAO inhibitor were to ingest foodstuffs containing tyramine, the tyramine could displace norepinephrine from its stores in the sympathetic nerves and increase blood pressure to a dangerously high level—a phenomenon called the “cheese effect.”

In patients who have autonomic failure with intact sympathetic nerves, the doctor might actually exploit the cheese effect, by prescribing a combination of tyramine with an MAO inhibitor.

There are two forms of MAO, MAO-A and MAO-B. MAO-B inhibitors are used to treat Parkinson disease. MAO-B inhibitors are much less likely to interfere with the breakdown of tyramine, because breakdown of tyramine in the gut and sympathetic nerves is mainly by MAO-A, and so patients treated with MAO-B inhibitors for Parkinson disease are not prone to the cheese effect.

Ganglion Blockade



Effects on blood pressure of injection of the ganglion blocker, trimethaphan, in a patient with pure autonomic failure (PAF), and in a patient with multiple system atrophy (MSA).

Trimethaphan and pentolinium are ganglion blockers—that is, they block transmission of nerve signals in the ganglia. The control signals are relayed in the ganglia by release of the chemical messenger, acetylcholine, which binds to nicotinic receptors on the post-ganglionic cells. Stimulation of the nicotinic receptors, such as by nicotine itself, increases post-ganglionic nerve traffic in both the parasympathetic and sympathetic nervous systems and releases adrenaline from the adrenal gland.

Trimethaphan and pentolinium block neuronal nicotinic receptors. By blocking the receptors, they inhibit the transmission of nerve impulses in the ganglia to the post-ganglionic nerves of the sympathetic and parasympathetic nervous systems.

Because of the blockade of transmission of nerve impulses in ganglia, trimethaphan and pentolinium produce clear effects on a variety of

body functions. When a person stands up, the ability to maintain blood pressure depends importantly on reflexes that tighten blood vessels by way of increased sympathetic noradrenergic nerve traffic. Trimethaphan and pentolinium therefore always produce a fall in blood pressure when the person is upright—orthostatic hypotension—and blunt or eliminate reflexive increases in heart rate. If the person were lying down at the time, the drugs produce smaller decreases in blood pressure.

Probably the most noticeable effect of ganglion blockade in someone who is lying down is a dry mouth. This is because of blockade of the parasympathetic nervous system, which is responsible for production of watery saliva.

In the ganglion blockade test, a ganglion blocker drug is given by vein at a dose calculated so as not to decrease the blood pressure excessively. The blood pressure and pulse rate are monitored frequently or continuously, and blood may be sampled from an indwelling catheter in an arm vein, for measurements of plasma levels of norepinephrine or other neurochemicals.

If a patient had autonomic failure due to a loss of sympathetic nerves, such as in pure autonomic failure, there would be no release of norepinephrine from the nerve terminals, because of the absence of the terminals. Ganglion blockade in such a patient would not decrease the blood pressure very much. But if a patient had autonomic and

baroreflex failure due to a brain disease in which there was an inability to regulate sympathetic nerve traffic to intact terminals, there might be ongoing, unregulated release of norepinephrine from the nerve terminals. Ganglion blockade in such a patient would decrease the blood pressure excessively, as in multiple system atrophy. The ganglion blockade test therefore can provide information about whether autonomic failure is associated with a loss of sympathetic nerve terminals or from failure of the brain to regulate sympathetic nerve traffic appropriately.

In some patients with long-term high blood pressure, the hypertension reflects an overall increase in the rate of nerve traffic in the sympathetic nervous system. This increases delivery of norepinephrine to its receptors in the heart and blood vessels, causing an increase in the output of blood by the heart (cardiac output) and tightening of blood vessels (vasoconstriction). By either or both mechanisms, the blood pressure is high because of the high rate of delivery of norepinephrine to its receptors. Some investigators have called this hypernoradrenergic hypertension. In a patient with hypernoradrenergic hypertension, infusion of trimethaphan or pentolinium would be expected to decrease the rate of norepinephrine release from the sympathetic nerves, and the extent of the fall in the plasma norepinephrine level would be related to the extent of the fall in blood pressure. In a patient with an equal amount of hypertension but with a normal rate of nerve traffic in the sympathetic noradrenergic system, ganglion blockade would not be expected to

decrease the blood pressure so much.

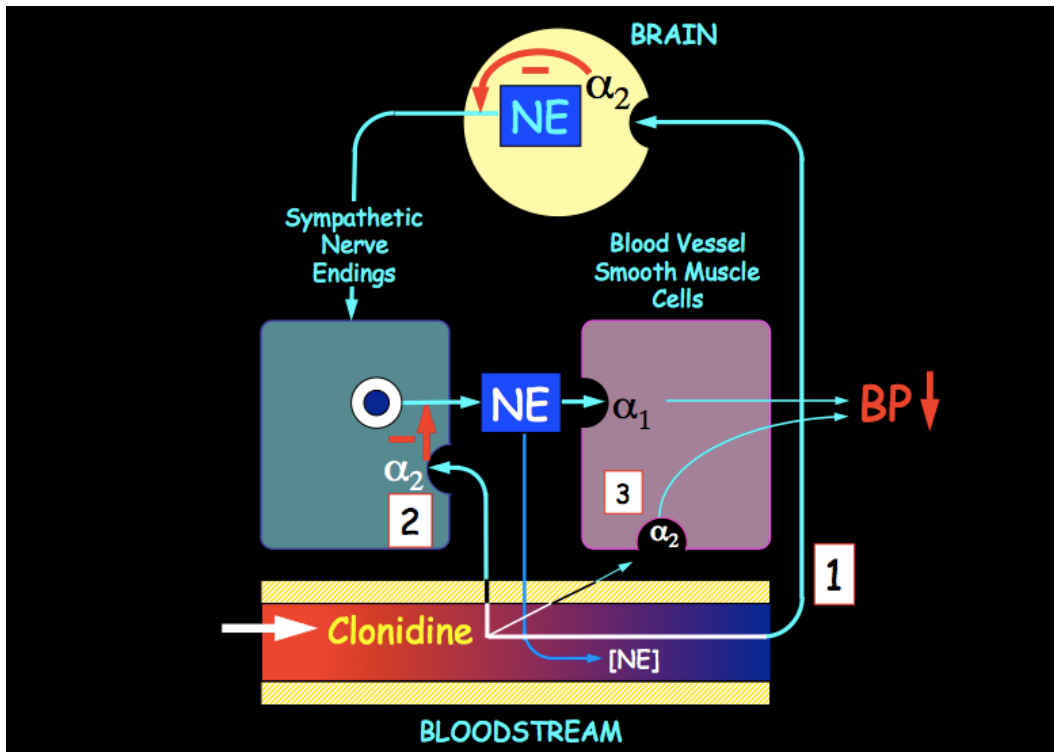
Because trimethaphan and pentolinium are potent blockers of the sympathetic and parasympathetic nervous systems, the drugs must be given at a carefully determined dose, by personnel who are well acquainted with their effects. If the dose were too high, then the blood pressure (especially the systolic blood pressure) could fall too much. The effects of ganglion blockade wear off fairly quickly—more quickly for trimethaphan.

Unfortunately, neither trimethaphan nor pentolinium is available commercially any more.

Clonidine

Clonidine (brand name Catapres™) is like the opposite of yohimbine. Clonidine stimulates alpha-2 adrenoceptors in the brain, in blood vessel walls, and on sympathetic nerve terminals. Clonidine decreases release of norepinephrine from sympathetic nerves and decreases blood pressure.

The clonidine suppression test is based on effects of the drug on blood pressure and on plasma levels of norepinephrine. If a patient had excessive activity of the sympathetic noradrenergic system, then clonidine would produce large decreases in blood pressure and plasma norepinephrine levels. Clonidine suppression testing therefore can identify long-term high blood pressure associated with increased



Clonidine decreases blood pressure by inhibiting sympathetic noradrenergic system (SNS) outflows and inhibiting norepinephrine release for a given amount of SNS traffic.

release of norepinephrine from sympathetic nerve terminals—
hypertension.

Clonidine suppression testing is used mainly to evaluate possible pheochromocytoma, a tumor that produces catecholamines. In pheochromocytoma plasma norepinephrine fails to decrease after clonidine administration, due to continuous, unregulated

norepinephrine secretion by the tumor.

Yohimbine

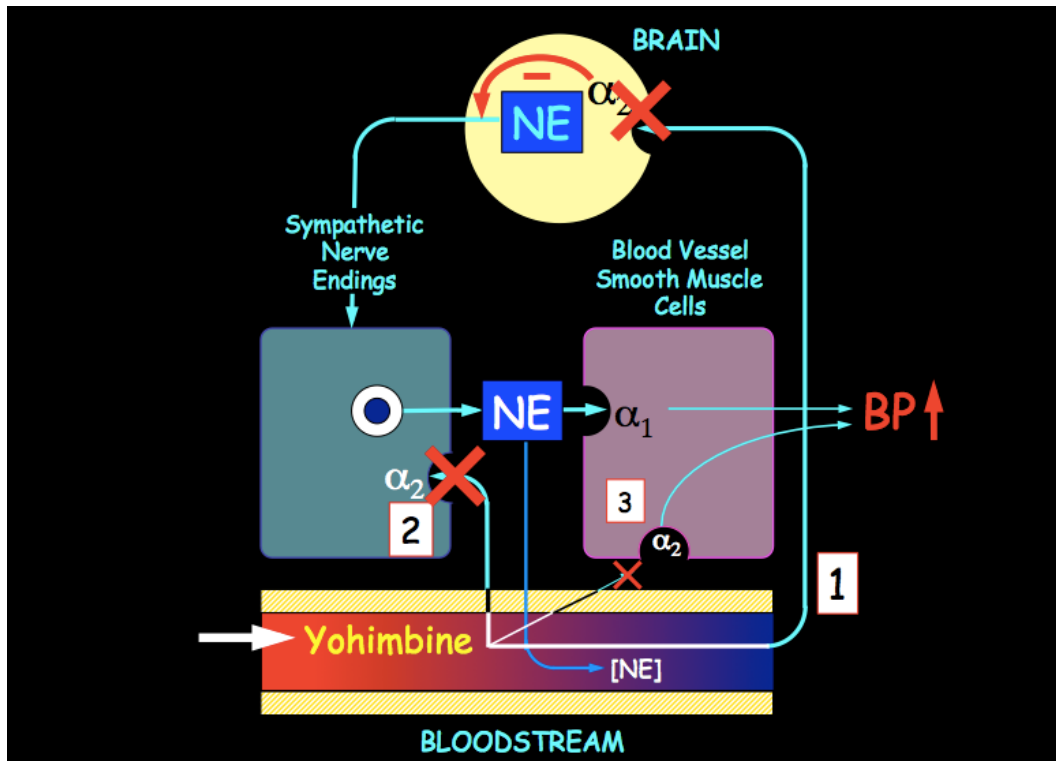
Yohimbine is a type of drug called an alpha-2 adrenoceptor blocker.

Alpha-2 adrenoceptors are receptors for norepinephrine that exist at high concentrations in certain parts of the brain, on sympathetic nerves, and in blood vessel walls.

When alpha-2 adrenoceptors in the brain are blocked, this increases sympathetic nerve traffic.

Alpha-2 adrenoceptors on sympathetic nerves act like a brake on norepinephrine release from the terminals. When alpha-2 adrenoceptors on sympathetic nerves are blocked, this increases the amount of norepinephrine release for a given amount of sympathetic nerve traffic.

By blocking alpha-2 adrenoceptors in the brain and on sympathetic nerve terminals, yohimbine releases norepinephrine from the terminals. The released norepinephrine binds to alpha-1 adrenoceptors in blood vessel walls, increasing the blood pressure.



Yohimbine increases blood pressure and plasma norepinephrine levels.

Yohimbine also blocks alpha-2 receptors on vascular smooth muscle cells, but this effect is minor compared to the effects in the brain and on sympathetic nerve terminals.

In the yohimbine challenge test, the drug is given by vein for several minutes or given by mouth as a single dose. Yohimbine given by vein is an investigational drug. The blood pressure and pulse rate are monitored frequently or continuously, and blood is sampled from an

indwelling catheter, for assays of plasma levels of norepinephrine and other neurochemicals.

Because of the blockade of alpha-2 adrenoceptors in the brain, infused yohimbine can produce any of several behavioral or emotional effects, which vary from person to person. Yohimbine can cause an increase in alertness or feelings such as anxiety or sadness, or, on the other hand, happiness or a sense of energy. Rarely, yohimbine infusion can evoke a panic attack; however, in my experience patients informed about the neurobehavioral effects of yohimbine and reassured that the effects are temporary do not report emotional changes as a result of the test.

Yohimbine infusion usually causes trembling, which sometimes is so severe that the teeth chatter. It also causes paleness of the skin, goose bumps, and piloerection (hair bristling), as if the person felt cold or distressed. Actually, the body temperature does not fall at all, and the person does not feel cold.

In hypertensive patients, the finding of a large increase in blood pressure coupled with a large increase in the plasma norepinephrine level during the yohimbine challenge test supports a diagnosis of hypernoradrenergic hypertension.

In patients who have decreased activity of the cell membrane norepinephrine transporter, or NET, when yohimbine releases

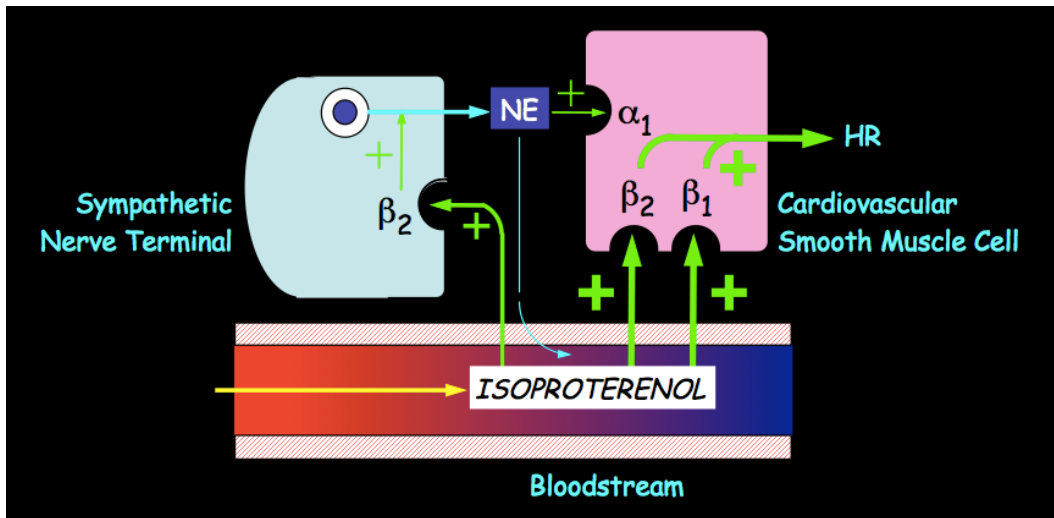
norepinephrine from the sympathetic nerves the released norepinephrine is not inactivated by reuptake of the norepinephrine back into the nerve terminals. This results in excessive delivery of norepinephrine to its receptors, both in the brain and outside the brain. In patients with NET deficiency, yohimbine produces large increases plasma norepinephrine and blood pressure. In NET deficiency yohimbine infusion can also evoke panic or chest pain or pressure that mimic the chest pain or pressure in coronary artery disease.

The yohimbine challenge test can provide useful information about whether autonomic failure is associated with a loss of sympathetic nerves or from failure of the brain to regulate sympathetic nerve traffic appropriately. In pure autonomic failure, yohimbine exerts relatively small effects on blood pressure or plasma norepinephrine levels, whereas in multiple system atrophy yohimbine produces large increases in both.

The yohimbine challenge test should be done only by personnel who are well acquainted with its effects in different forms of dysautonomia.

Isoproterenol

The isoproterenol infusion test can help identify causes of abnormal heart rate or inability to tolerate prolonged standing.



Isoproterenol stimulates beta-adrenoceptors.

Isoproterenol (brand name Isuprel™) stimulates beta-adrenoceptors. Because of this action, isoproterenol has several effects in the body. Stimulation of beta-adrenoceptors in the heart increases the rate and force of the heartbeat and increases the output of blood by the heart per minute (cardiac output). Stimulation of beta-adrenoceptors in the bronchioles, the small airway tubes in the lungs, opens them and therefore can reverse acute asthma attacks. Stimulation of beta-adrenoceptors in the liver converts stored energy in the form of glycogen to immediately available energy in the form of glucose. Stimulation of beta-adrenoceptors in blood vessel walls of skeletal muscle relaxes the blood vessels, and this decreases the resistance to blood flow in the body as a whole (total peripheral resistance). Stimulation of beta-adrenoceptors on sympathetic nerves increases the release of norepinephrine.

Isoproterenol is infused by vein as part of diagnostic testing for a few types of dysautonomias.

The isoproterenol infusion test can help identify causes of abnormal heart rate or inability to tolerate prolonged standing.

In the hyperdynamic circulation syndrome, the patient has a relatively fast pulse rate, high cardiac output, a variable blood pressure that tends to be high, susceptibility to panic or anxiety attacks, and improvement by treatment with the beta-adrenoceptor blocker, propranolol. The same holds true for many relatively young patients with early, borderline hypertension. Such patients have excessive increases in pulse rate in response to isoproterenol given by vein.

Patients with the postural tachycardia syndrome (POTS) can have a fast pulse rate even when they are lying down. In POTS patients isoproterenol can produce excessive increases in heart rate or evoke panic.

Isoproterenol given by vein is also sometimes used as part of tilt table testing in patients with chronic fatigue syndrome. During upright tilting, infusion of isoproterenol can bring on a rapid fall in blood pressure or loss of consciousness, converting a negative tilt table test to a positive tilt table test. This brings up the issue of how frequently

a healthy person might have one of these reactions in response to isoproterenol infusion while tilted—a false-positive result.

Isoproterenol releases norepinephrine from sympathetic nerves. Patients with a form of dysautonomia associated with a loss of sympathetic nerves would be expected to have a blunted increase in the plasma norepinephrine level in response to isoproterenol. This is what happens, for instance, in Parkinson disease with orthostatic hypotension.

The effects of isoproterenol wear off rapidly within minutes of stopping the infusion. The drug does not enter the brain well, and so there are usually few if any behavioral or emotional responses. Isoproterenol can increase the rate or depth of respiration, produce trembling, or bring on abnormal heart rhythms or abnormal heartbeats. These side effects disappear rapidly after the drug is stopped.

Glucagon

Glucagon is one of the body's three main hormones regulating glucose levels.

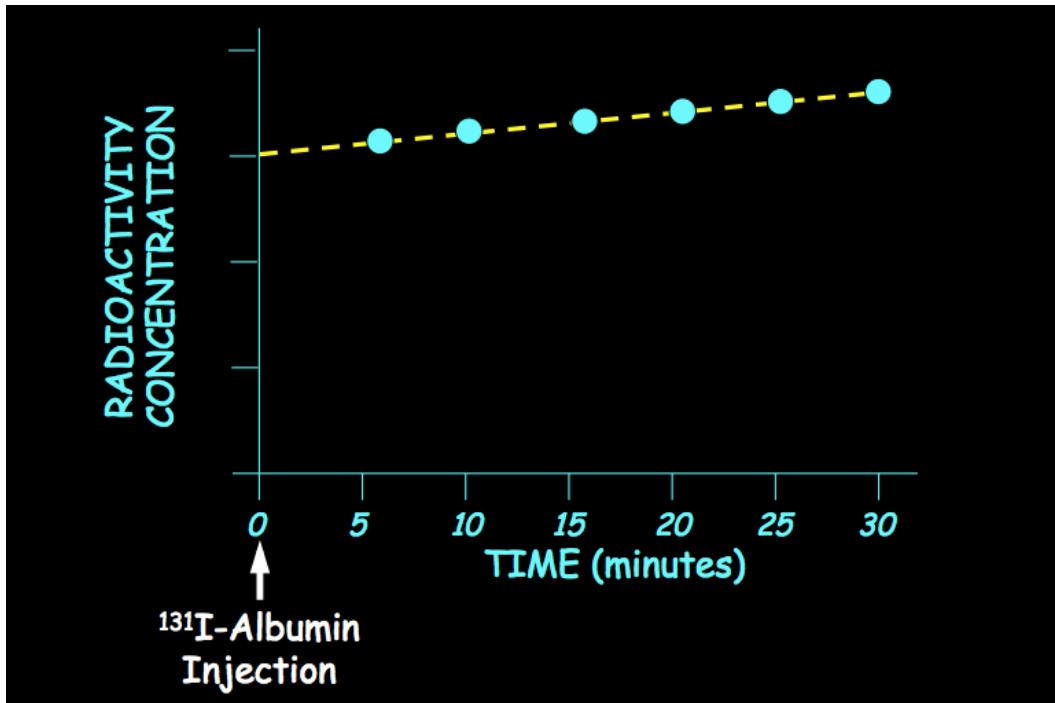
When given i.v. as a bolus, glucagon stimulates release of adrenaline from the adrenal medulla. In patients with an adrenal gland tumor that makes catecholamines (pheochromocytoma, or “pheo”), glucagon

challenge testing can produce a large increase in blood pressure. Glucagon challenge testing is also used in the evaluation of patients who seem to have a pheo clinically but who don't actually harbor the tumor. These patients are thought to have "pseudopheochromocytoma," or "pseudopheo." The condition can resemble forms of dysautonomia such as postural tachycardia syndrome, arterial baroreflex failure, or hyperdynamic circulation syndrome. Glucagon administration in pseudopheo patients can evoke a large increase in plasma adrenaline levels. This constitutes a positive glucagon challenge test.

¹³¹I-Albumin to Measure Blood Volume

Blood volume is the total volume of blood in the body. Most of the blood volume is in veins. When a person stands up, blood pools in the legs, pelvis, and abdomen due to gravity. If the blood volume were low, then because of gravitational blood pooling there would be less blood returning to the heart to pump to body organs including the brain, and the person could feel lightheaded or faint.

In patients with chronic orthostatic intolerance, measurement of blood volume may be indicated, since if the blood volume were low, a drug such as fludrocortisone and a high salt diet might benefit the patient, by increasing blood volume.



In the blood volume test, blood volume is calculated from the concentration and amount of a drug in the bloodstream.

There are different ways to measure blood volume. A commercially available test is based on i.v. injection of albumin that is tagged with a trace amount of radioactive iodine (^{131}I -albumin). Albumin is the main protein in the blood. In the ^{131}I -albumin blood volume test, an exact, known amount of ^{131}I -albumin is injected into a vein. Most of the injected ^{131}I -albumin stays in the bloodstream. Blood is drawn, and the concentration of ^{131}I in the plasma is measured. By definition, the concentration of a substance is the amount of the substance per unit of volume. Since the amount of ^{131}I injected is known, and the

plasma concentration of ^{131}I is measured in the laboratory, by algebra the plasma volume is the ^{131}I concentration divided by the amount of injected ^{131}I . From the plasma volume divided by the hematocrit (the percent of the blood that is red blood cells), the blood volume is then calculated.

Because the concentration of ^{131}I - albumin in the blood actually may change slightly over time, (such as by leakage out of the blood vessels), blood is sampled at several time points, and the concentration that is estimated to be present in the blood immediately after injection is used for the calculation of blood volume.

BIOCHEMICAL TESTS

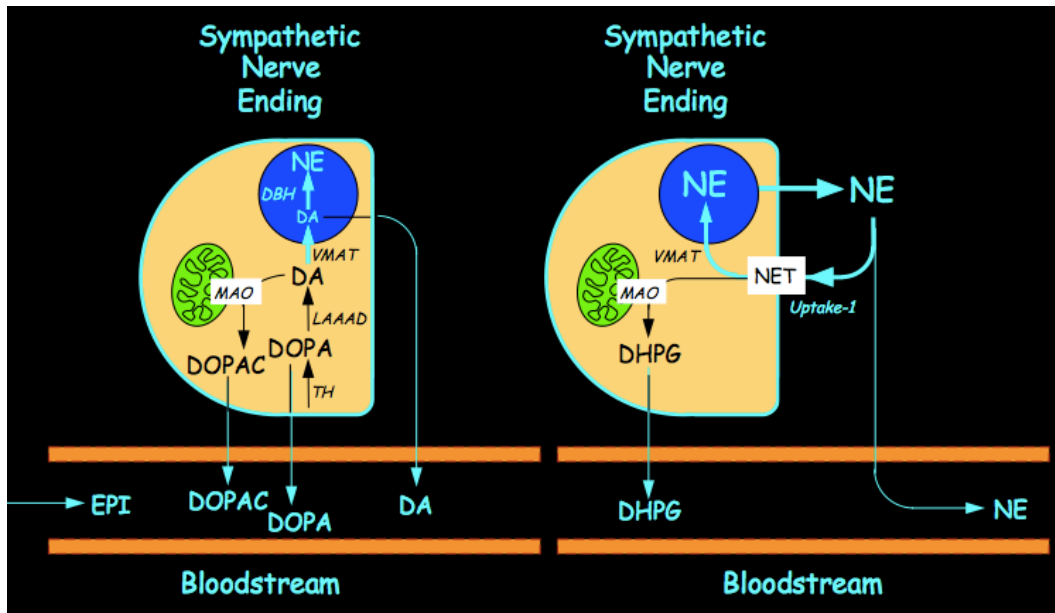
Neurochemical tests of autonomic nervous system function mainly examine activities of the sympathetic noradrenergic system (SNS) and the sympathetic adrenergic system (SAS).

This is because the main chemical messengers of these systems, norepinephrine and epinephrine (adrenaline), can be measured in the plasma, whereas the main chemical messenger of the parasympathetic nervous system, acetylcholine, undergoes rapid breakdown by an enzyme and cannot be measured in the plasma.

The Cat Comes Back

Human plasma normally contains at least 6 catechols. Three are the catecholamines dopamine, norepinephrine, and adrenaline (epinephrine, EPI). Another is DOPA (also known as levodopa), which is the precursor of the catecholamines and the immediate product of the enzymatic rate-limiting step in catecholamine biosynthesis. Two others are dihydroxyphenylglycol (DHPG), which is the main neuronal metabolite of norepinephrine, and dihydroxyphenylacetic acid (DOPAC), which is the main neuronal metabolite of dopamine.

The actual chemical, catechol, doesn't exist in the body. I use the



Human plasma contains at least 6 catechols—the catecholamines NE, EPI, and DA, the catecholamine precursor DOPA, the DA metabolite DOPAC, and the NE metabolite DHPG.

term, catechols, to mean chemicals that have the catechol structure in them, which as you recall looks like the head of a cat.

Dopamine depletion in a particular brain pathway causes the movement disorder in Parkinson disease; however, plasma dopamine is unrelated to dopamine in the brain. At least some of plasma dopamine is derived from vesicles in sympathetic noradrenergic nerves, presumably because of exocytotic release from the vesicles

before the dopamine has had a chance to be converted to norepinephrine. Plasma dopamine levels normally are very low—as little as a few picograms (a millionth of a millionth of a gram) per milliliter.

Norepinephrine is the main major chemical messenger of the sympathetic noradrenergic system (SNS) and is also a neurotransmitter in the brain. Plasma and cerebrospinal norepinephrine levels are related to each other. Since there is an effective blood-brain barrier for catecholamines, this relationship indicates an association between norepinephrine release in the brain and SNS outflow.

Epinephrine, or adrenaline, is the hormone of the sympathetic adrenergic system (SAS). Adrenaline is produced from norepinephrine by the actions of a few co-factors and the enzyme, phenylethanolamine-N-methyltransferase, abbreviated PNMT. S-Adenosyl-methionine acts as the methyl group donor for the reaction. Importantly, cortisol is trophic for the actions of PNMT. This is a key site of linkage between two of the body's main stress systems.

Norepinephrine is produced from dopamine, by the enzyme, dopamine-beta-hydroxylase, or DBH. DBH is localized to the storage vesicles in the sympathetic noradrenergic nerves. Therefore, in order to produce norepinephrine, dopamine in the cytoplasm must be taken up into the vesicles. In the vesicles the dopamine is converted to

norepinephrine via DBH.

Plasma Norepinephrine (NE)

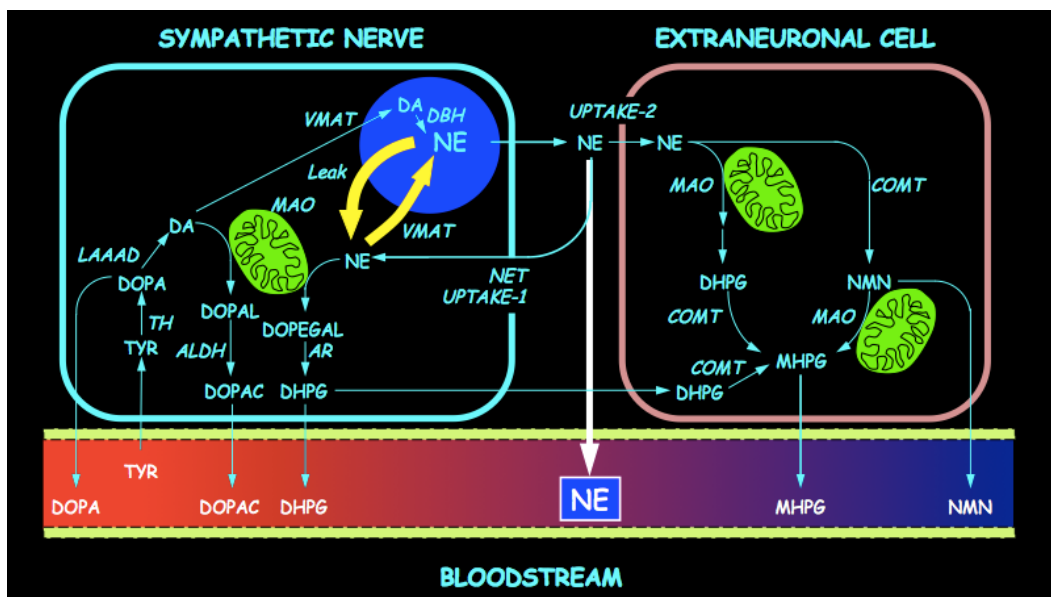
Since norepinephrine is the main chemical messenger of the sympathetic noradrenergic system, doctors have often used the plasma norepinephrine level as an index of sympathetic nervous system “activity” in the body as a whole. In people who are resting lying down, plasma NE levels normally range from about 100 to about 500 pg/mL.

Plasma norepinephrine is used to test the part of the sympathetic nervous system that regulates the heart and blood vessels—the sympathetic noradrenergic system.

The relationship between the rate of sympathetic nerve traffic and the concentration of norepinephrine in the plasma is complex and indirect and is influenced by many factors such as commonly used drugs and activities of daily life. The blood sample should be obtained under carefully controlled or monitored conditions, and the plasma norepinephrine level should be interpreted by an expert.

Here is a brief description of some of the complexities involved:

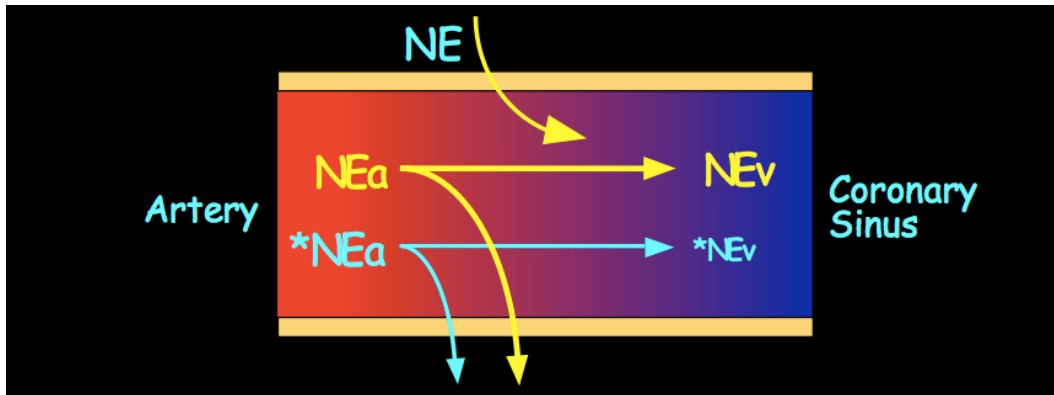
First, only a small percent of the norepinephrine released from sympathetic nerves actually makes its way into the bloodstream.



Determinants of plasma norepinephrine levels

Most is recycled back into the nerve terminals, by the Uptake-1 process mediated by the cell membrane norepinephrine transporter, or NET. This means that a person might have a high plasma norepinephrine level, despite a normal rate of sympathetic nerve traffic, if the NET were blocked by a drug or weren't working right.

Second, the plasma norepinephrine level is determined not only by the rate of entry of norepinephrine into the plasma but also by the rate of removal of norepinephrine from the plasma. Norepinephrine is cleared from the plasma extremely rapidly (half-time about 1.5 minutes). This means that a person might have a high plasma



One can estimate the rate of entry of norepinephrine (NE) into the bloodstream by infusing tracer-labeled NE and sampling arterial and venous blood.

norepinephrine level because of a problem with the ability to remove norepinephrine from the plasma, such as in kidney failure.

One way to estimate the rate of norepinephrine entry into the bloodstream (“spillover”) after taking plasma clearance into account is based on i.v. infusion of a tracer-labeled form of norepinephrine. The person’s own norepinephrine, which is not labeled, dilutes the tracer-labeled norepinephrine. The more dilution of the tracer, the greater the norepinephrine spillover.

The tracer dilution technique cannot distinguish norepinephrine release from norepinephrine reuptake as determinants of spillover. Simultaneous measurement of levels of tracer-labeled DHPG can do this. To the extent neuronal uptake of the tracer-labeled

norepinephrine were decreased, formation of tracer-labeled DHPG would be decreased. If there were an increase in norepinephrine release without a change in reuptake, then the norepinephrine spillover would be increased without a change in formation of tracer-labeled DHPG.

Third, norepinephrine is produced in sympathetic nerve terminals by the action of three enzymes, along with other required chemicals such as vitamin C, vitamin B6, and oxygen. In addition, norepinephrine is produced in, stored in, and released from tiny bubble-like “vesicles” in sympathetic nerves. For norepinephrine to be produced in the vesicles requires another transporter, called the “vesicular monoamine transporter,” or “VMAT.” A problem with any of these enzymes, co-factors, or the VMAT can result in decreased norepinephrine production and therefore a low plasma norepinephrine level, regardless of the rate of sympathetic nerve traffic.

Fourth, the plasma norepinephrine level usually is measured in a blood sample drawn from a vein in the arm. Because the skin and skeletal muscle in the forearm and hand contain many sympathetic nerves, the plasma norepinephrine level in blood from an arm vein is determined not only by the amount of norepinephrine release from sympathetic nerves in the body as a whole but also by the amount of release locally in the forearm and hand.

Fifth, the plasma norepinephrine level varies depending on the posture

of the person at the time of blood sampling (the level normally approximately doubles within 5 minutes of standing up from lying down), the time of day (highest in the morning), whether the person has been fasting, the temperature of the room, dietary factors such as salt intake, and any of a large number of commonly used over-the-counter and prescription drugs or herbal remedies.

Levels of a variety of chemicals are related to the production and fate of norepinephrine and provide information about functioning of the sympathetic noradrenergic system.

Plasma Epinephrine (EPI)

Compared to the plasma norepinephrine level, which is complexly and indirectly related to sympathetic noradrenergic system “activity” in the body as a whole, the plasma epinephrine (adrenaline) level is a fairly direct indicator of activity of the sympathetic adrenergic system (adrenomedullary hormonal system).

Plasma adrenaline (epinephrine) is used to test the sympathetic adrenergic system (SAS).

Nevertheless, some of the same factors that make interpreting plasma norepinephrine levels difficult can complicate interpreting plasma

adrenaline levels. When the forearm vascular resistance is high, EPI in arterial blood is extracted in passage through tissues of the arm, and in this setting the EPI level in the arm venous plasma substantially underestimates the level in the arterial plasma.

A large number of common and difficult to control life experiences influence activity of the sympathetic adrenergic system. These include drugs, alterations in blood glucose levels (such as after a meal), body temperature, posture, and emotional distress.

An additional problem is technical. Adrenaline is a very powerful hormone. Predictably, the plasma adrenaline level normally is very low—so low that it is often below the limit of detection of commercial laboratory assays. In a healthy person lying down, plasma EPI levels can be as low as a few pg/mL.

Other chemicals besides adrenaline can interfere with the measurement. This can especially be a problem in people who drink a lot of coffee, even if it is decaffeinated, because of high plasma levels of a chemical called dihydrocaffeic acid, which can mimic adrenaline in some assay procedures. Because of these issues, it is important that blood sampling and chemical assays for plasma adrenaline levels be carried out by experienced and expert personnel.

Plasma and CSF DHPG

3,4-Dihydroxyphenylglycol (DHPG, DOPEG) is the main neuronal metabolite of norepinephrine (NE). In people who are at rest lying down, plasma DHPG averages about 500-1,200 pg/mL.

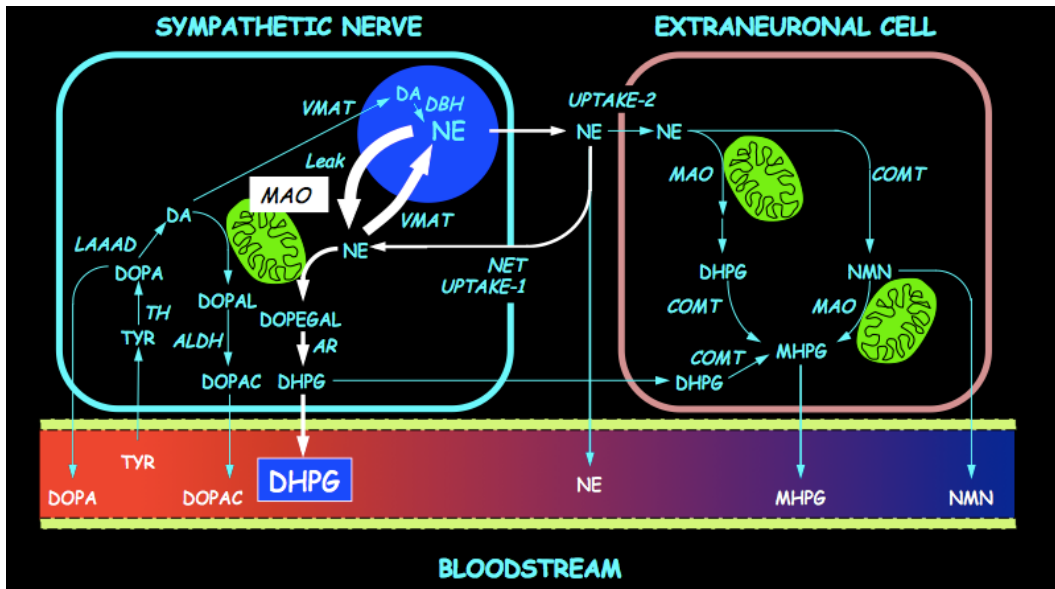
Plasma DHPG levels provide important supplementary information with which to interpret plasma NE levels.

Plasma DHPG levels have somewhat different determinants from plasma NE levels. Plasma NE levels are determined importantly by exocytotic release in response to sympathetic nerve traffic and by neuronal reuptake via the cell membrane norepinephrine transporter (NET) via the Uptake-1 process.

Under resting conditions, plasma DHPG levels are determined mainly by the net leakage of NE stored in vesicles and by monoamine oxidase (MAO) activity in sympathetic noradrenergic nerves.

In the setting of increased sympathetic noradrenergic system (SNS) activity, the increase in NE release results in an increase in plasma NE levels. This is what happens, for instance, during standing up (orthostasis). Plasma NE levels normally double within 5 minutes of standing up from lying down (normal increase at least 60%). Plasma DHPG levels also increase, due to reuptake of some of the released NE, but by a much smaller percent than the increase in plasma NE.

In the setting of loss of SNS terminals, such as occurs in pure

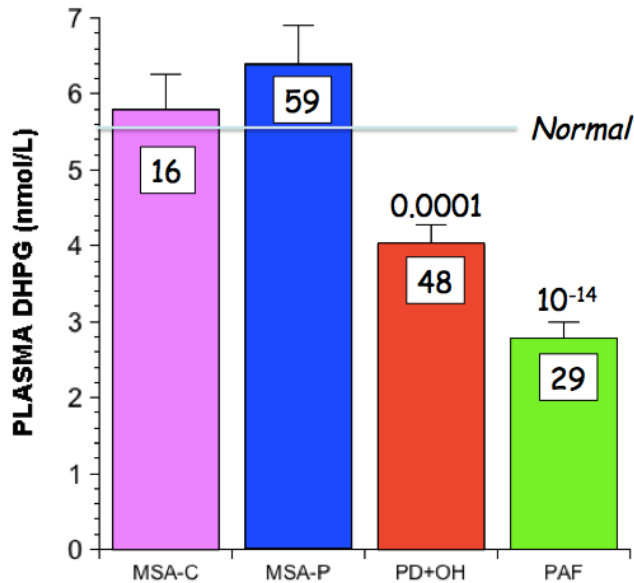


Plasma DHPG levels are determined importantly by stores of norepinephrine in sympathetic noradrenergic nerves.

autonomic failure, there is a loss of NE stores, and plasma DHPG levels are decreased. Meanwhile, because of compensatorily increased SNS traffic in the residual nerves, plasma NE levels can be maintained.

Thus, in pure autonomic failure, which is known to be associated with substantial SNS denervation, plasma DHPG levels are decreased more than plasma NE levels.

Plasma DHPG provides a better measure of sympathetic



Plasma DHPG is normal in MSA, often decreased in PD +OH, and almost always decreased in PAF

innervation in the body as a whole than does plasma norepinephrine.

In the setting of NET inhibition, such as due to tricyclic anti-depressants, some amphetamines, or cocaine, for the same amount of NE release there is less reuptake of the released NE. This means that plasma DHPG levels are decreased more than are plasma NE levels. During orthostasis, the increase in plasma NE is larger than the increase in plasma DHPG, and the plasma NE:DHPG ratio is high. This is what is found in postural tachycardia syndrome due to NET deficiency.

The parkinsonian form of multiple system atrophy (MSA-P) can be very difficult to distinguish from Parkinson disease with orthostatic hypotension (PD+OH). Plasma DHPG can provide a neurochemical clue to the correct diagnosis. This is because sympathetic noradrenergic innervation is generally intact in MSA-P, and the orthostatic hypotension is mainly from baroreflex failure. In PD+OH there is also a generalized loss of sympathetic noradrenergic nerves. Although plasma NE levels usually are normal in PD+OH, plasma DHPG levels often are low, reflecting the loss of NE stores.

Since DHPG is a deaminated metabolite of NE, MAO inhibition decreases plasma DHPG levels without affecting plasma NE levels. The form of MAO in sympathetic noradrenergic nerves is MAO-A. Even so, treatment with an MAO-B inhibitor can decrease plasma DHPG levels.

CSF DHPG provides a valuable neurochemical means to detect loss of NE stores in the brain. In this regard CSF DHPG is more informative than CSF NE.

Plasma DOPA

DOPA (3,4-dihydroxyphenylalanine, levodopa) is the immediate product of the rate-limiting enzymatic step in the synthesis of the catecholamines—hydroxylation of tyrosine catalyzed by tyrosine

hydroxylase (TH).

Human plasma normally contains a higher concentration of DOPA than of any of the catecholamines. Plasma DOPA is determined complexly, but one determinant is TH activity. Since TH outside the brain is concentrated in sympathetic noradrenergic nerves, patients with severe sympathetic noradrenergic denervation can have decreased plasma DOPA levels. On the other hand, in diseases involving decreased norepinephrine synthesis but normal TH activity, such as Menkes disease, plasma DOPA levels are increased compared to those of DHPG.

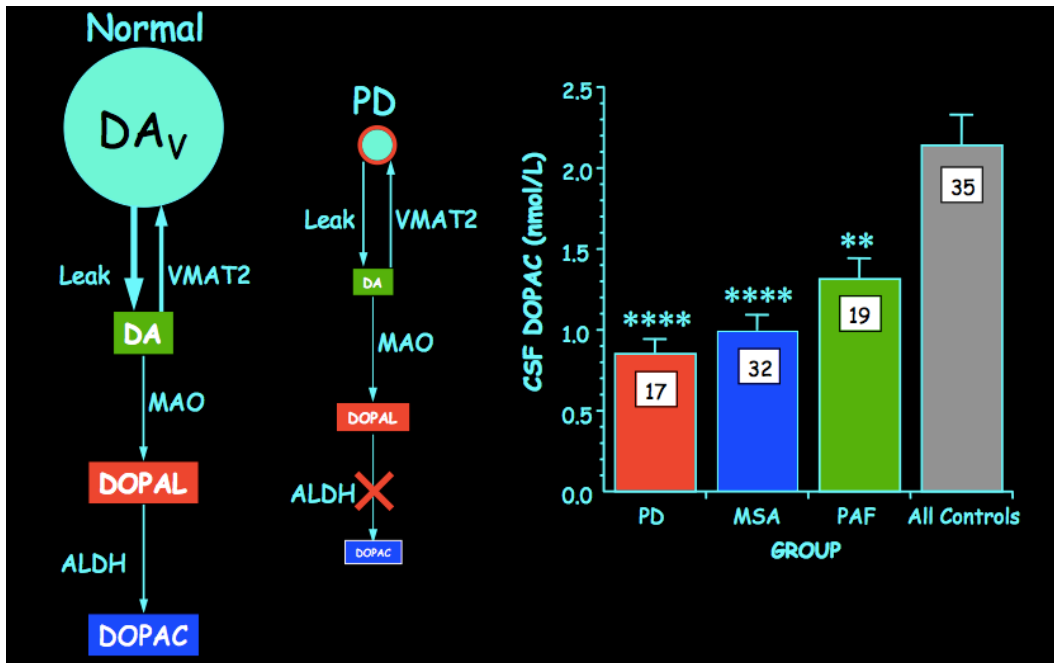
Plasma levels of DOPA average about 1,000-2,000 pg/mL. Patients treated with levodopa, such as for Parkinson disease, can have plasma DOPA levels a thousand times higher than found endogenously.

Plasma and CSF DOPAC

DOPAC (3,4-dihydroxyphenylacetic acid) is the main neuronal metabolite of dopamine. Plasma DOPAC levels are much higher than plasma dopamine levels, averaging about 1,000-2,000 pg/mL.

Since DOPAC is a deaminated metabolite, patients on monoamine oxidase inhibitors can have decreased plasma DOPAC levels.

DOPAC levels in cerebrospinal fluid (CSF) provide a neurochemical



CSF levels of DOPAC are low in Parkinson's disease and related disorders.

“window” on the status of dopaminergic innervation in the brain. In parkinsonism from any cause, CSF DOPAC is decreased.

Low CSF DOPAC may reflect any of several abnormalities. First there is a loss of dopaminergic neurons. Second there can be a vesicular storage defect in the residual dopaminergic neurons, limiting the amount of dopamine stores and therefore the amount of substrate for monoamine oxidase to act on. Third is a decrease in activity of the enzyme, aldehyde dehydrogenase (ALDH). After dopamine in the cytoplasm is deaminated by monoamine oxidase, the immediate

product is an aldehyde, 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is ALDH to form DOPAC. Decreased ALDH activity in dopaminergic neurons in the brain would be expected to be associated with low CSF DOPAC levels, and post-mortem studies have noted decreased putamen ALDH activity in Parkinson's disease and in the parkinsonian form of multiple system atrophy. MAO inhibition would be expected to decreased CSF DOPAC, as would increased activity of catechol-O-methyltransferase, which converts DOPAC to homovanillic acid in non-neuronal cells.

Antibody Tests

One mechanism by which autonomic nerves could be harmed is by auto-immunity. The idea is that an organism expresses a protein that is normally expressed in the autonomic nervous system. The immune system raises an antibody to that protein, which damages or interferes with the function of the nerves.

Probably the most well characterized form of auto-immune attack is autoimmune autonomic neuropathy from a circulating antibody to the neuronal nicotinic receptor. Since ganglionic neurotransmission depends on this receptor, autoimmune autonomic neuropathy can manifest with decreased function of any component of the autonomic nervous system. In autoimmune autonomic ganglionopathy (AAG), the attack is sufficiently severe and generalized to cause all components of the autonomic nervous system to fail—a

“pandysautonomia.” In AAG, the titer of the antibody to the neuronal nicotinic receptor correlates with the severity of the patient’s symptoms and signs.

Cancer cells can produce antibodies to proteins expressed by autonomic nerves (“paraneoplastic syndrome”). Anti-Hu antibodies (also known as Type 1 anti-neuronal nuclear antibody, ANNA-1) are especially common in small cell lung cancers.

A variety of infectious diseases can result in autonomic neuropathies, such as mononucleosis, herpes simplex, and Coxsackie B.

Lambert-Eaton myasthenic syndrome is an autoimmune disorder of neuromuscular transmission characterized by antibodies directed against presynaptic, voltage-gated calcium channels that impair acetylcholine release. This syndrome is most commonly associated with symptoms and signs of parasympathetic nervous system failure.

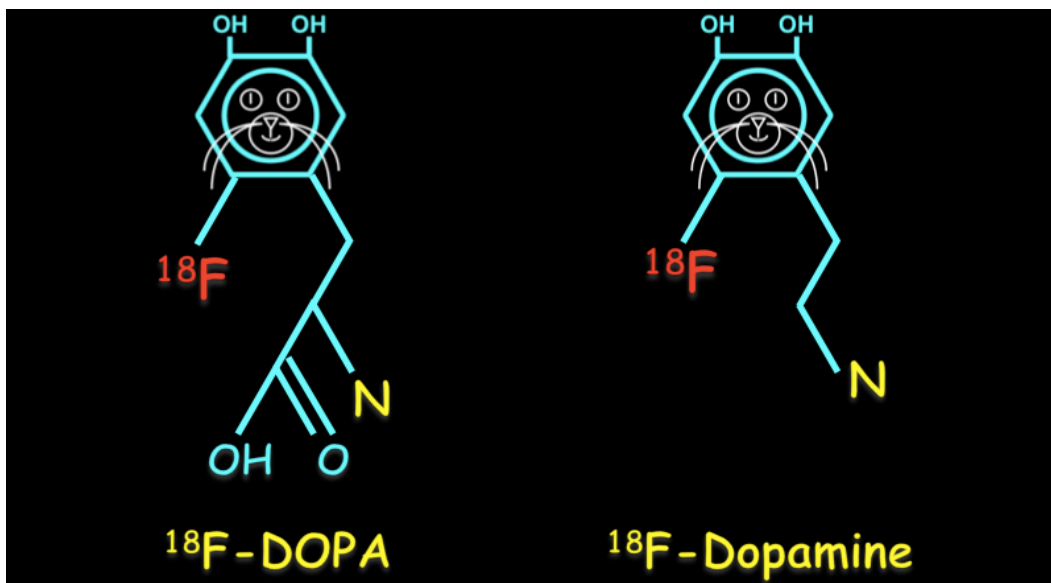
Several diseases can include autonomic neuropathy that may have an autoimmune mechanism, such as diabetes, Guillain-Barré syndrome, Sjogren’s syndrome, lupus, and amyloidosis. In general, there is no specific test to identify the specific offending antibody.

Other antibodies are included in commercially available panels. Some associated with autonomic neuropathies are antinuclear antibody and Rheumatoid factor.

It should be noted that the presence of an antibody, such as to the neuronal nicotinic receptor, does not mean that it is pathogenic and causes or contributes to dysautonomia. It can be very difficult to make this determination with confidence. One way to assess this possibility is by plasma exchange. In this procedure, the patient's blood is drawn into a machine that separates the cells from the plasma, removes the plasma, and infuses the patient's cells back into the patient, along with saline, albumin, and electrolytes. Plasma exchange temporarily decreases circulating levels of all antibodies. Rapid improvement in the patient's symptoms and signs would indicate that one or more antibodies are pathogenic.

NEUROIMAGING TESTS

Neuroimaging is a way to see nervous system tissue. Cardiac sympathetic neuroimaging, to visualize the sympathetic noradrenergic innervation in the heart, can be especially valuable in distinguishing PD with orthostatic hypotension from the parkinsonian form of multiple system atrophy. Cardiac sympathetic neuroimaging can also distinguish Lewy body dementia from Alzheimer's disease. Brain striatal neuroimaging, by ^{18}F -DOPA PET scanning, is valuable to confirm loss of putamen dopamine terminals, a common feature of parkinsonism, whether from Parkinson's disease, the parkinsonian form of multiple system atrophy, or progressive supranuclear palsy.



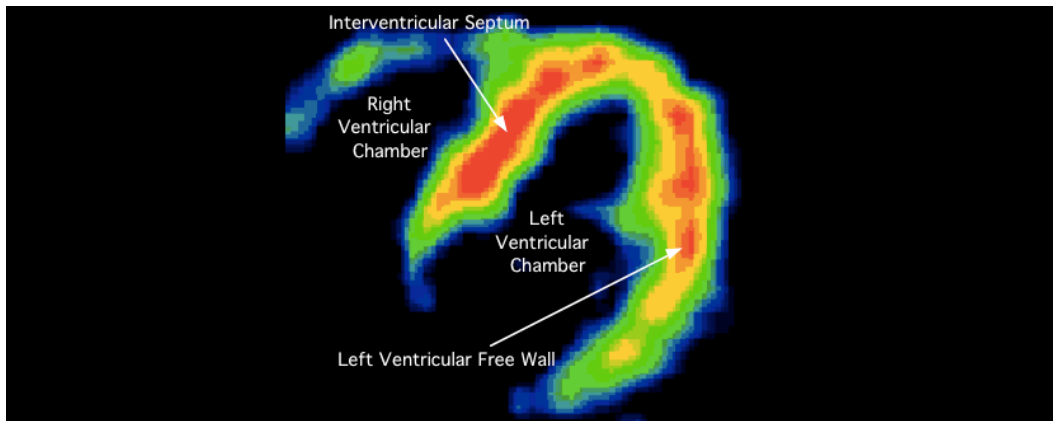
^{18}F -DOPA and ^{18}F -dopamine are catechols.

Cardiac Sympathetic Neuroimaging

Sympathetic nerves in the heart travel with the coronary arteries that deliver blood to the heart muscle. The nerves then dive into the muscle and form mesh-like networks that surround the heart muscle cells. Because neuroimaging tests have a limit of resolution of a few millimeters, the imaging does not show individual nerves but gives a general picture, and since the nerves are found throughout the heart muscle, the picture looks very much like a scan of the heart muscle. The radioactive drugs used for imaging the sympathetic nerves in the heart are given by injection into a vein, and they are delivered to the heart muscle by way of the coronary arteries. One must be able to distinguish decreased radioactivity in the scan due to loss of sympathetic nerves from decreased radioactivity due to blockage of a coronary artery, because either nerve loss or coronary blockage would lead to the same lack of radioactivity in the heart muscle. Centers that carry out sympathetic neuroimaging therefore often do two scans in the same test, one scan to see where the blood is going and one to see where the sympathetic nerves are.

MIBG Scanning

Sympathetic neuroimaging is available in few centers In the United

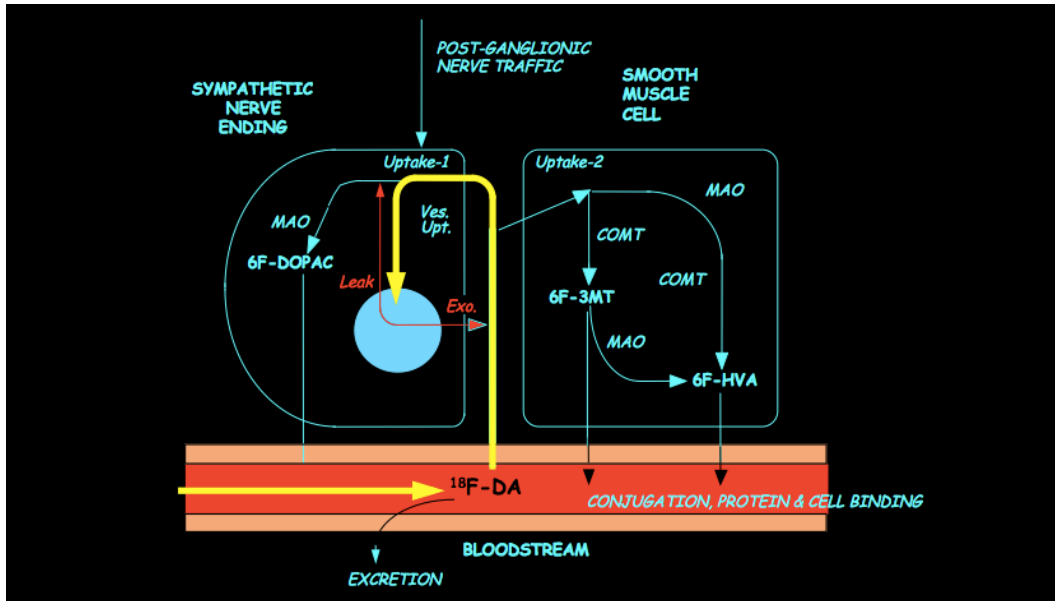


¹⁸F-Dopamine PET scanning can visualize the sympathetic nerves in the heart.

States but is done routinely at many centers in Europe and Japan. Worldwide the most commonly used sympathetic neuroimaging agent is ¹²³I- metaiodobenzylguanidine, or ¹²³I-MIBG. ¹²³I-MIBG is a radioactive form of a drug that is taken up by sympathetic nerves, making them visible on a nuclear medicine scanner.

¹²³I-MIBG scanning is used fairly commonly to evaluate possible pheochromocytoma, a tumor that is a rare but important cause of high blood pressure; however, ¹²³I-MIBG scanning to examine the sympathetic nerve supply to the heart as part of autonomic function testing is still considered to be a research tool and therefore is not currently covered by medical insurance plans.

In common forms of dysautonomia such as postural tachycardia

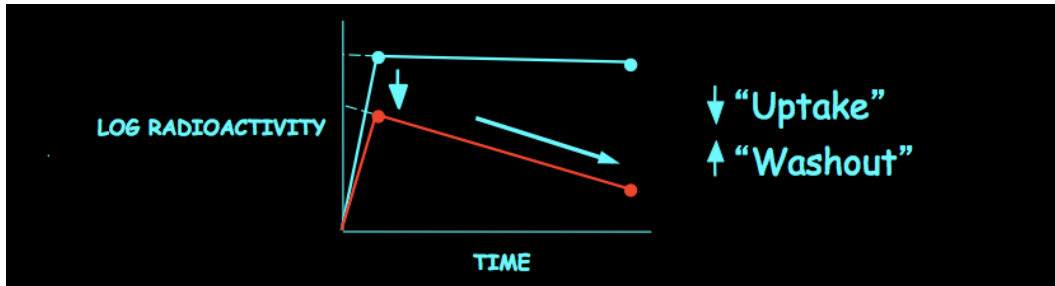


^{18}F -Dopamine injection leads to radiolabeling of vesicles in sympathetic nerves.

syndrome the problem is not a loss of the sympathetic nerves but a change in activity or function of those nerves. Whether ^{123}I -MIBG scanning can provide information about cardiac sympathetic function is a research question.

Fluorodopamine PET Scanning

At the National Institutes of Health's Clinical Center, in Bethesda, Maryland, another sympathetic neuroimaging agent has been developed, which is 6- ^{18}F fluorodopamine, or ^{18}F -dopamine. This is



The dynamics of radioactivity over time may provide information about the functioning of the sympathetic nerves in the heart.

a radioactive form of the catecholamine, dopamine.

After injection of ^{18}F -dopamine into a vein the drug is taken up by sympathetic nerves, and the radioactivity is detected by a special type of scanning procedure called positron emission tomographic scanning, or “PET scanning.”

Imagine you had a radioactive object in a box. You could determine if there were something radioactive inside by using a detector, such as a Geiger counter. Now suppose you had a large number of little Geiger counters all around the box. Tomographic scans are two-dimensional images, or slices. Tomographic slices would allow you to see what was inside the box at any level. If the object were small, most of the slices would be empty. Eventually, at the level of the object, you would see an image of the object in the slice.

A positron emitter is a type of radioactive substance that releases a short-lived form of radiation that can penetrate the body and reach detectors outside it, enabling construction of a PET scan. Other scans in nuclear medicine use a somewhat different source of radioactivity, but the idea is about the same.

Fluorodopamine is similar structurally to two of the biochemicals of the sympathetic nervous system, norepinephrine and adrenaline. Just as some radioactive chemicals get taken up by bone, producing a bone scan, or get taken up by the brain, producing a brain scan, fluorodopamine gets taken up by sympathetic nerves, and the result is a scan of the sympathetic nervous system. For instance, we know that fluorodopamine gets taken up very readily in the heart walls, since there are so many sympathetic nerves there. Because there are so many sympathetic nerves in the heart, PET scans of the heart after injection of fluorodopamine basically look like images of the heart itself. One can easily make out the main pumping muscle (left ventricular myocardium), the septum between the left and right ventricles, and the left and right ventricular chambers that contain the blood the heart pumps.

Different forms of dysautonomia result in remarkably different pictures of the sympathetic nerves in the heart by fluorodopamine PET scanning. Probably the most striking pictures occur in diseases where there is a loss of sympathetic nerves, such as in pure autonomic failure and in Parkinson disease, because even when the blood flow to

the heart muscle is normal, there is no heart visible in the PET scan.

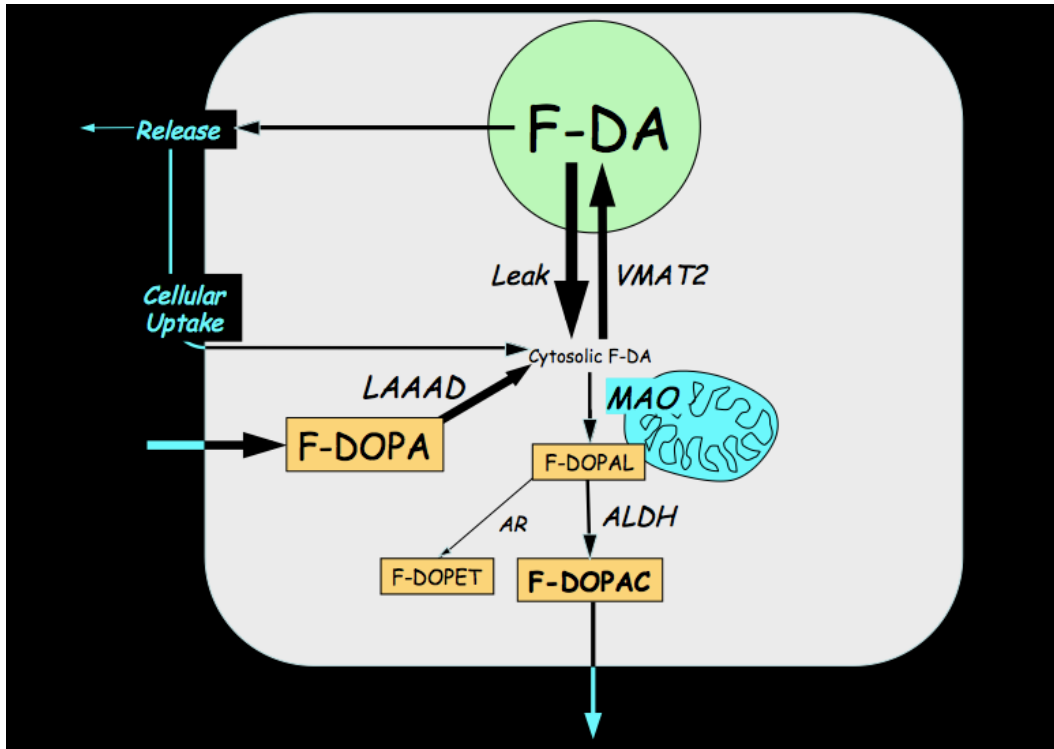
Analysis of the amount of radioactivity in the heart over time can provide information about how the sympathetic nerves are functioning. This is a matter of research interest now.

Striatal Dopaminergic Neuroimaging

^{18}F -Dopamine, which is used for cardiac sympathetic neuroimaging, does not pass through the “blood-brain barrier.” This means that ^{18}F -dopamine PET scanning cannot reveal abnormalities in different structures in the brain. ^{18}F -DOPA, on the other hand, can penetrate the blood-brain barrier. Inside the brain, the ^{18}F -DOPA is converted to ^{18}F -dopamine, and the radioactivity is concentrated in regions that store catecholamines such as dopamine and norepinephrine.

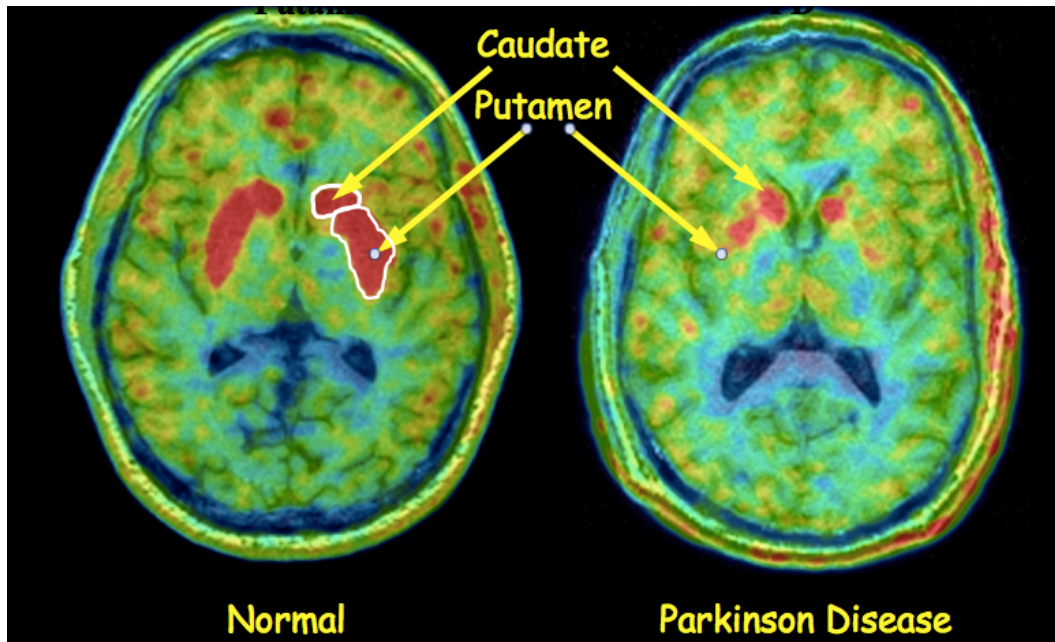
After i.v. injection of ^{18}F -DOPA, relatively high concentrations of radioactivity accumulate in the basal ganglia of the brain. The striatum is a major site of loss of dopamine terminals in Parkinson’s disease and related disorders. When dysautonomia occurs in the setting of a neurodegenerative disease, ^{18}F -DOPA PET scanning can help determine if the disease is associated with loss of dopamine terminals in the striatum.

The striatum in the brain consists of the caudate and putamen. In a



^{18}F -DOPA striatal neuroimaging is based on conversion of radioactive ^{18}F -DOPA to ^{18}F -dopamine and uptake of the ^{18}F -dopamine into vesicles, making them show up on a PET scan.

^{18}F -DOPA PET scan the striata on the two sides of the brain look like slugs, or like a sad clown's eyes. The clown's beady eyes correspond to the head of the caudate. The "eye liner" corresponds to the putamen, which is the major site of damage in Parkinson's disease. In the scan, the eye liner seems washed away.



Special scans of the brain can show the damage in diseases associated with dysautonomia, such as Parkinson's disease.

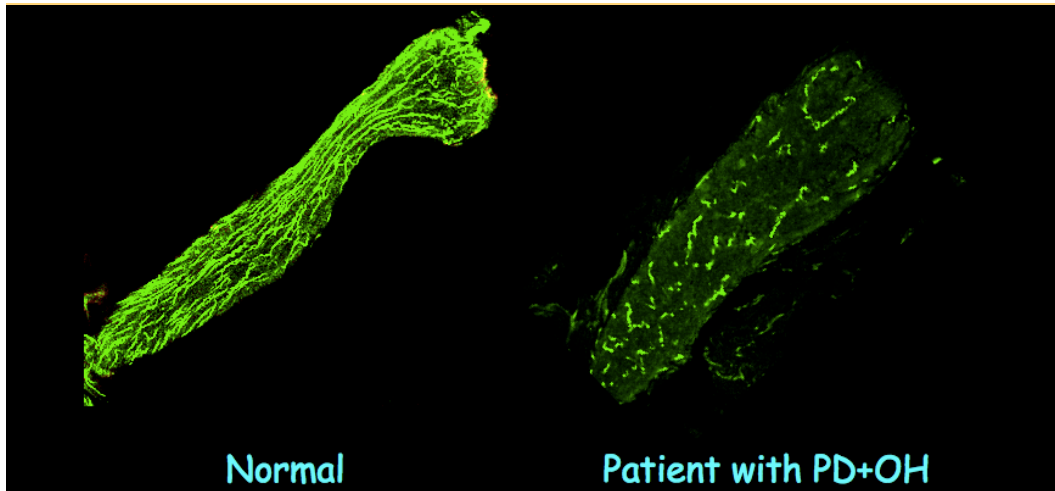
There are several other imaging agents that can be used like ^{18}F -DOPA to visualize abnormalities of the nigrostriatal dopamine system in the brain. A related type of scan is called a “DAT” scan. DAT stands for the cell membrane dopamine transporter. Since transporters for dopamine are found on the terminals in the striatum, a DAT scan can detect loss of dopamine terminals such as in Parkinson's disease.

SKIN BIOPSIES

The skin possesses multiple types of nerves. Nerves in the skin that sense touch and vibration are relatively large and conduct impulses quickly. Thin, slow-conducting, non-myelinated nerve fibers in the skin can be identified by a chemical called PGP 9.5. Sympathetic cholinergic and sympathetic noradrenergic fibers, as well as slow-conducting sensory fibers, are stained by PGP 9.5.

The sweat glands in the skin receive sympathetic cholinergic nerve fibers. These are post-ganglionic, non-myelinated, slow-conducting fibers that release acetylcholine. The acetylcholine binds to muscarinic receptors, evoking sweat secretion. In skin biopsy samples, one can identify the sympathetic cholinergic fibers by their contents of vasoactive intestinal peptide (VIP) or choline acetyltransferase (ChAT).

The hair follicles have small muscles attached to them called *arrector pili* muscles. The *arrector pili* muscles are responsible for the hair standing up, or piloerection, when you are exposed to cold or when you are emotionally distressed. The *arrector pili* muscles receive sympathetic noradrenergic nerve fibers. These are post-ganglionic, non-myelinated, slow-conducting fibers that release norepinephrine. The norepinephrine binds to alpha-adrenoceptors, and the hair stands up. In skin biopsy samples, one can identify the sympathetic



Loss and fragmentation of sympathetic noradrenergic nerve fibers seen by tyrosine hydroxylase staining in a patients with Parkinson disease and orthostatic hypotension (PD+OH). (Images courtesy of C. Gibbons, N. Wang, & R. Freeman)

noradrenergic fibers by their contents of dopamine-beta-hydroxylase (DBH) or tyrosine hydroxylase (TH).

Usually skin biopsy samples contain tiny blood vessels. The walls of arterioles receive sympathetic noradrenergic nerve fibers, which can be identified by dopamine-beta-hydroxylase (DBH) or tyrosine hydroxylase (TH) staining.

Since the *arrector pili* muscles and the walls of the arterioles receive only sympathetic noradrenergic nerves, PGP 9.5 staining can identify sympathetic noradrenergic nerves specifically by their location.

GENETIC TESTS

Most forms of dysautonomia do not run in families, but some do. Knowledge about inherited predispositions to dysautonomias is expanding rapidly. As of this writing there are relatively few genetic tests for dysautonomias.

Inherited forms of dysautonomia are rare.

Familial Dysautonomia

The most well known inherited dysautonomia is familial dysautonomia (FD). FD runs in families of Ashkenazi extraction. The cause is a mutation of the gene, IKBKAP. Genetic screening for FD is now available.

DBH Deficiency

A very rare cause of orthostatic hypotension is deficiency of the enzyme, dopamine-beta-hydroxylase (DBH). This enzyme is required to produce norepinephrine. Genetic testing may be indicated in patients with orthostatic hypotension who have biochemical test results that are consistent with DBH deficiency.

NET Deficiency

A very rare cause of postural tachycardia syndrome (POTS) is

mutation of the gene for the cell membrane norepinephrine transporter (NET). Although POTS is relatively common, POTS from this genetic cause is extremely rare. Genetic screening for NET deficiency may be indicated for POTS patients who have biochemical or neuroimaging test results that are consistent with NET deficiency.

Menkes Disease

Menkes disease is a rare disease of copper metabolism. Because dopamine-beta-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine, is a copper enzyme, Menkes disease involves a form of dysautonomia due to decreased norepinephrine production.

Genetic mutation of a copper ATPase causes Menkes disease. The gene is located on the X-chromosome. This means that Menkes disease is transmitted as an X-linked recessive trait. The patients are virtually always boys, since males have only 1 X chromosome. The mother, with 2 X chromosomes, is a carrier. If a woman has given birth to a baby with Menkes disease, then chances are 50:50 that if she has another son the baby will be affected. Genetic testing for the mutated gene can be done in the fetus.

WHICH TESTS ARE DONE WHERE?

Few hospitals in the United States carry out comprehensive autonomic function testing.

Physiological tests such as measurements of heart rate responses to the Valsalva maneuver are readily available, neuropharmacologic tests such as the QSART and skin biopsies are done in several specialized autonomic function testing centers, neurochemical tests such as plasma norepinephrine and adrenaline levels are done in fewer centers, and as of this writing neuroimaging tests are rarely available in the United States, because third party payers don't cover them. At this point there are only a few genetic tests for particular forms of dysautonomia, such as familial dysautonomia.

At a minimum the battery of autonomic function tests at a given medical center should be able to identify abnormalities of regulation of the circulation by the sympathetic noradrenergic system, sweating by the sympathetic cholinergic system, and heart rate by the parasympathetic nervous system.

Other types of testing would depend on the particular problem the patient is facing. For instance, for complete assessment of dysautonomia in a patient with evidence of a neurodegenerative disease such as Parkinson disease, neuroimaging should be available to examine dopamine centers in the brain and the supply of

sympathetic nerves in the heart; while for assessment of postural tachycardia syndrome, measurement of blood volume may be indicated.

As part of tilt table testing for the evaluation of autonomically mediated syncope, I think it is important to track plasma adrenaline and norepinephrine levels. This is the only way to detect differential changes in activities of the sympathetic adrenergic and sympathetic noradrenergic systems—sympathoadrenal imbalance—which seems to be a key factor in fainting reactions.

There is now an autonomic Rare Diseases Clinical Research Consortium. The founding centers in the Consortium are at the Vanderbilt University School of Medicine in Nashville, TN; the Mayo Clinic in Rochester, MN; the New England Deaconess Medical Center in Boston, MA; the NYU Medical Center in New York City, NY; and the National Institute of Neurological Disorders and Stroke in Bethesda, MD. These centers routinely carry out autonomic function testing as part of the research under the Consortium.

There is also a newly inaugurated certification program in autonomic disorders, under the United Council of Neurological Subspecialties (UCNS). Physicians who are certified under the program are knowledgeable about autonomic function testing.