Tests for Dysautonomias

There are four types of tests doctors use to diagnose dysautonomias—physiological, neuropharmacologic, neurochemical, and neuroimaging.

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.

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

Neurochemical tests involve measuring levels of body chemicals, such as the *catecholamines, norepinephrine* and *epinephrine,* either under resting conditions or in response to *physiological* or *neuropharmacologic* manipulations.

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

This section describes examples of each type of test. Each type has its own advantages and disadvantages. Most centers that carry out *autonomic function testing* use more than one type of test, but none use all of the tests described in this section.

Physiological tests usually are simple, quick, painless, and safe. The main problem with them is that there are always several steps between the brain's directing a change in nerve traffic in the *autonomic nervous system* and the *physiological* changes that are supposed to measure the *autonomic* changes. As a result, the results of *physiological* tests are always complex and **indirect**, and they may or may not identify a problem correctly.

Neuropharmacologic tests are relatively simple and quick, but they depend on drug effects on how the patient feels or how the body functions. This means that there always is at least some risk of **side effects**. In addition, neuropharmacologic tests are somewhat complex and indirect. 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 system* wasn't involved with the patient's high blood pressure.

Neurochemical tests involve measurements of levels of compounds such as *norepinephrine* in body fluids such as *plasma*. These tests can be done while the patient is at

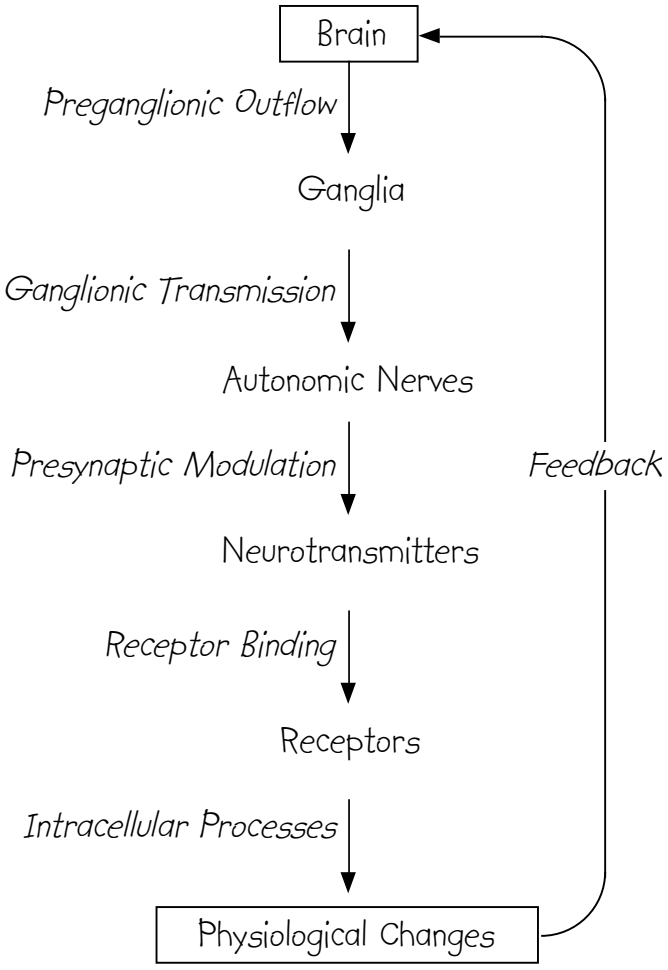
rest lying down, during a *physiological* manipulation such as exercise or tilting on a *tilt-table,* or during a *neuropharmacologic* manipulation such as blockade of *sympathetic nerve traffic* by a drug. *Neurochemical* tests themselves are safe, but the type of body fluid sampling, such as *arterial blood sampling* or *cerebrospinal fluid* sampling after a *lumbar puncture,* can involve some risk.

A major disadvantage of *neurochemical* testing is that there is **no test of parasympathetic nervous system activity.** This is because *acetylcholine,* the chemical messenger of the *parasympathetic nervous system,* is broken down by enzymes almost as soon as it enters body fluids such as the *plasma.*

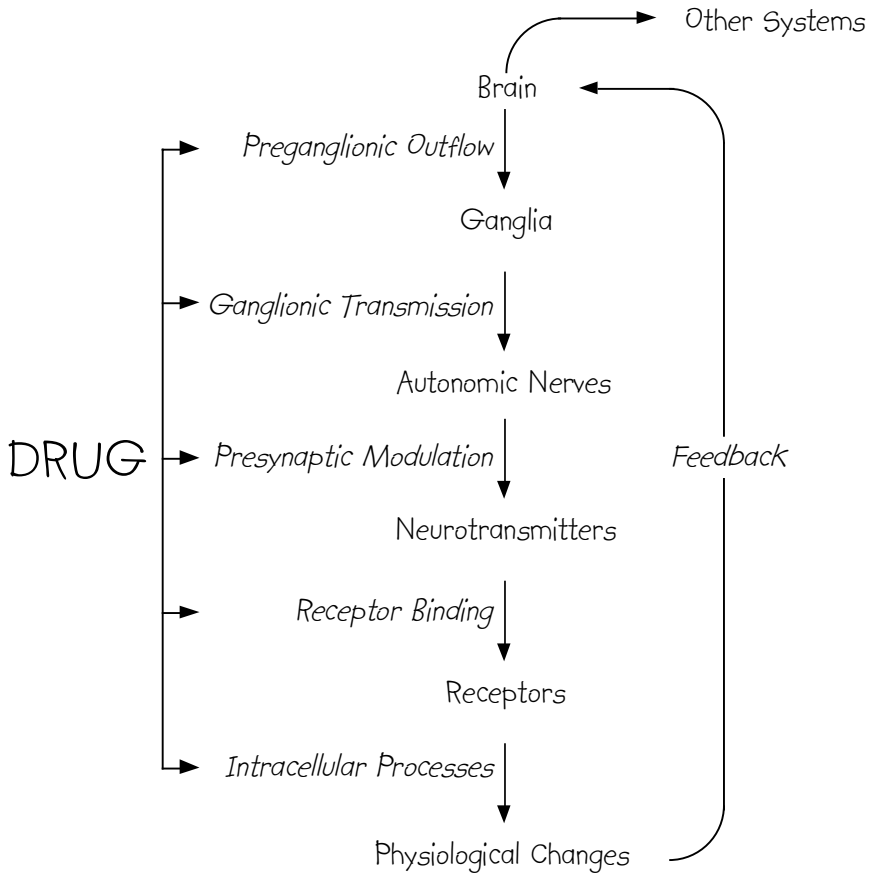
Neurochemical testing based on *plasma norepinephrine* levels also can be problematic, because those levels are determined not only by the rate of entry of *norepinephrine* into the plasma but also the rate of removal *(clearance)* of *norepinephrine* from the *plasma.* In addition, *plasma norepinephrine levels* are determined complexly by a variety of processes in the *sympathetic nerve terminals. Neurochemical* testing by *plasma norepinephrine* levels requires a carefully **controlled testing situation** and **expert technical analysis** and **interpretation.** Few clinical laboratories measure *plasma* levels of *catecholamines* such as *norepinephrine* and *epinephrine,* and **laboratories vary** in the validity of the assay methods they use.

Neuroimaging tests, which are relatively new, involve actually depicting the *autonomic nerve supply* in body

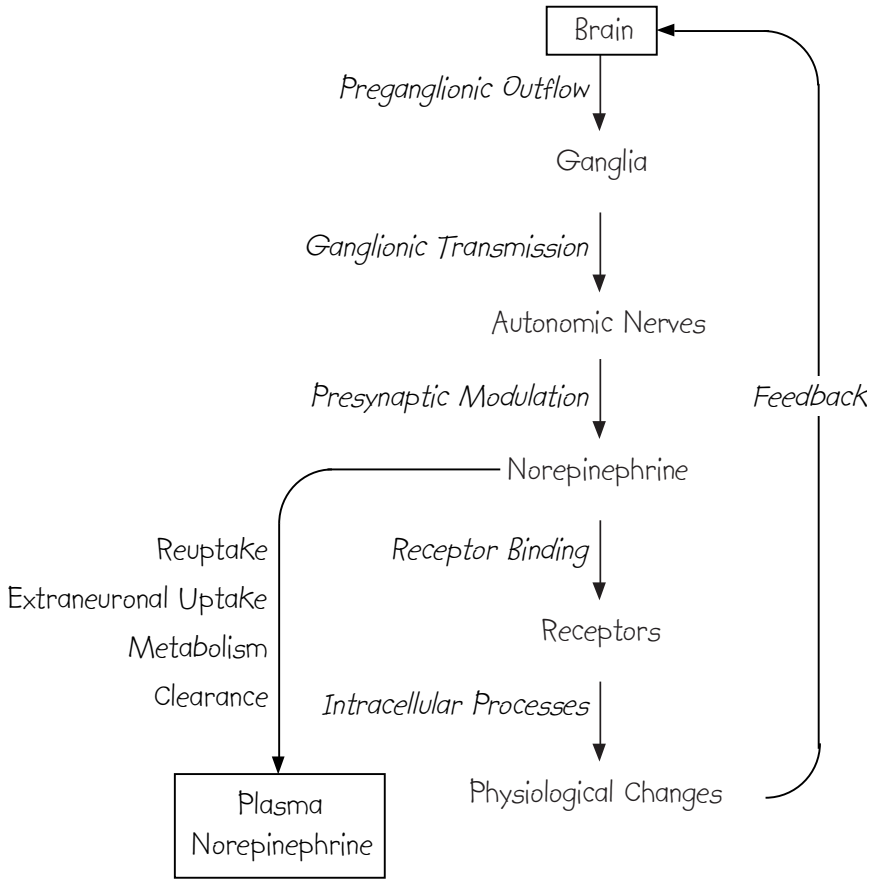
organs such as the heart. As yet there is no accepted neuroimaging test to visualize *parasympathetic nerve terminals. Sympathetic neuroimaging* is done in relatively **few centers,** and although this type of testing can produce striking images of the *sympathetic innervation* of the heart, this provides **anatomic** information about whether *sympathetic nerve terminals* are present, and it is still unclear whether *sympathetic neuroimaging* can provide information about whether those terminals are functioning normally or not.



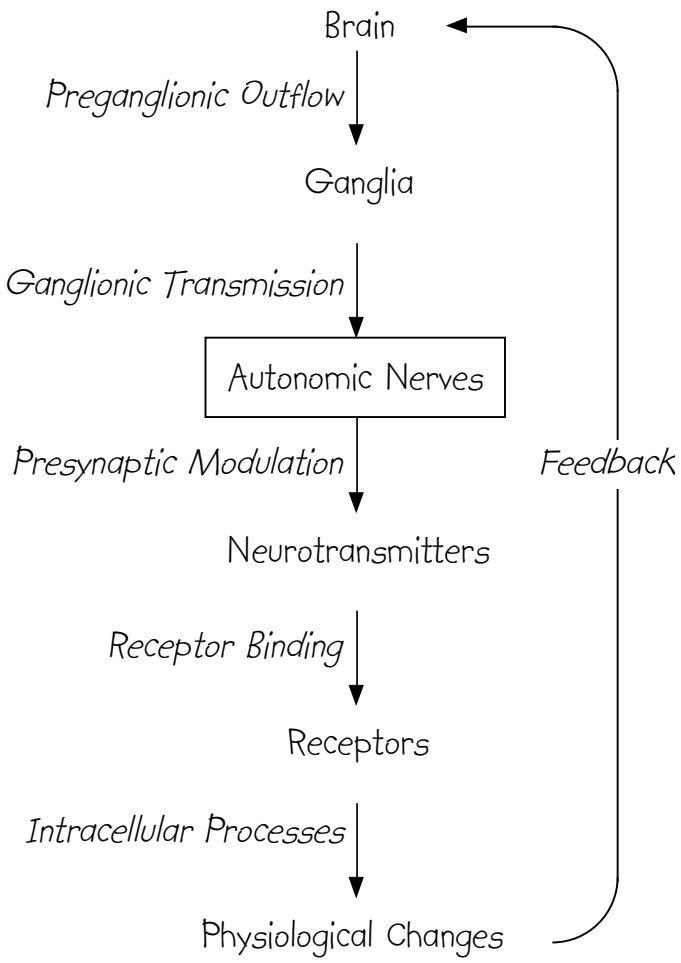
Physiological tests involve measurement of a body function, such as pulse rate or blood pressure.



Neuropharmacologic tests involve using a drug that affects a function of the nervous system.



Neurochemical tests involve measuring a chemical produced in the nervous system, such as norepinephrine, the chemical messenger of the sympathetic nervous system.



Neuroimaging tests involve visualizing part of the nervous system, such as the autonomic nervous system.

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.

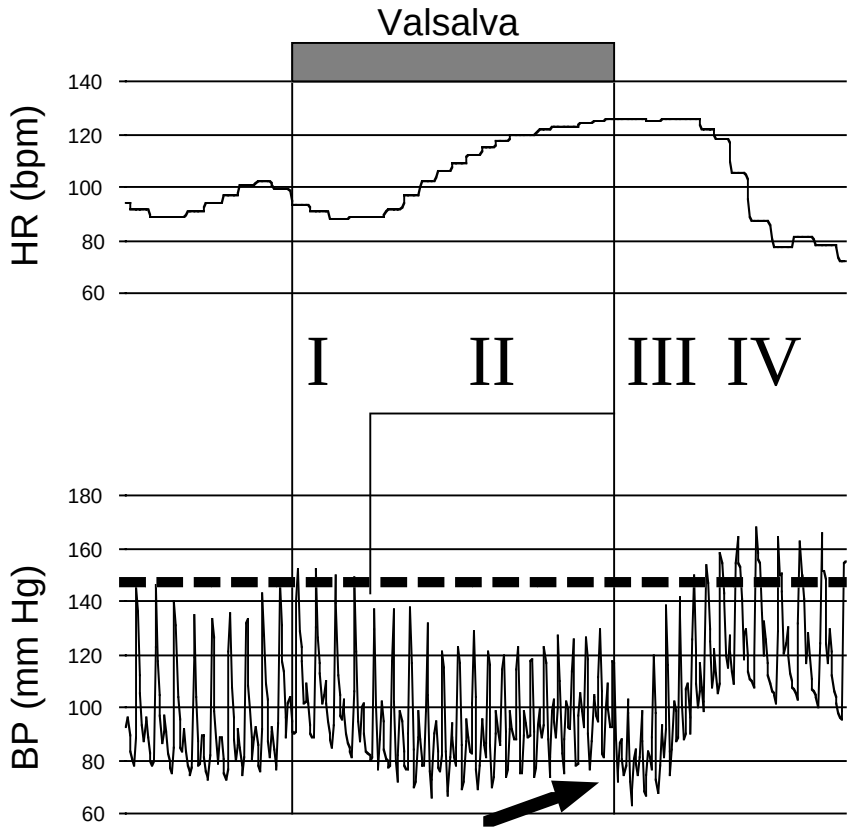
The maneuver consists of blowing against a resistance for several seconds and then relaxing. Often the patient blows into a tube connected to a blood pressure gauge, moving the gauge's needle to a particular pressure and keeping the needle there for 10-15 seconds.

The instant the patient begins to blow, the sudden increase in chest and abdominal pressure forces blood out of the chest and down the arms. This increases blood pressure briefly (Phase I of the maneuver). The increase in blood pressure in Phase I is mechanical and not part of a reflex.

Soon afterwards, however, the amount of blood ejected by the heart with each beat *(stroke volume)* plummets, because the straining decreases entry of blood from the

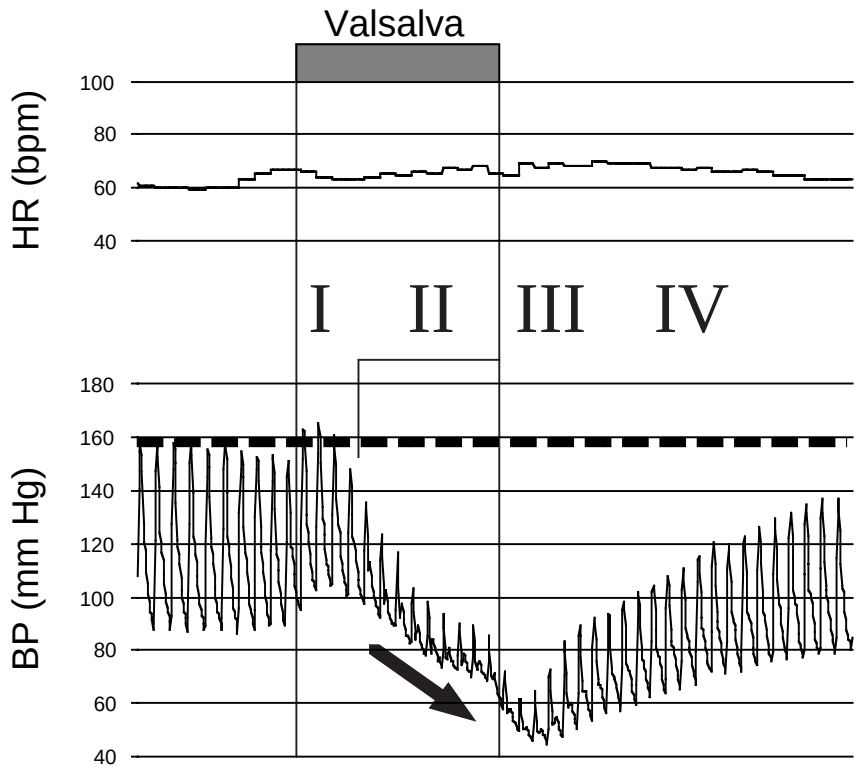
veins into the heart. Blood pressure progressively falls (Phase II). The brain immediately senses this fall, due to decreased input to the brain from stretch receptors *(baroreceptors)* in the walls of the heart and major blood vessels. The brain directs a rapid increase in outflows in the *sympathetic nervous system* to the blood vessels and a rapid decrease in outflow in the *parasympathetic nervous system* to the heart. The increase in *sympathetic nerve traffic* leads to more release of *norepinephrine* from the nerve terminals, and the released *norepinephrine* tightens blood vessels throughout the body. The *total peripheral resistance* to blood flow in the body goes up, just like tightening the nozzle at the end of a garden hose increases the pressure in the hose. Therefore, normally, at the end of Phase II the blood pressure increases from its minimum value, even though the amount of blood ejected by the heart remains low.

When the patient relaxes at the end of the maneuver, briefly the blood pressure falls (Phase III)—a mirror image of the brief increase in Phase I. Blood rushes back into the chest, and within a few heartbeats the heart ejects this blood. The blood pressure increases (Phase IV). Since the blood vessels are constricted, the normal amount of filling of the constricted vessels produces an overshoot of blood pressure, just as pressure in a garden hose attains higher levels if one turns on the faucet with the nozzle tightened. Finally, in response to this Phase IV overshoot of blood pressure, *sympathetic nervous system* outflow to blood vessels falls and *parasympathetic* outflow to the heart increases. This causes a rapid return of blood pressure and heart rate to normal.



Normal blood pressure (BP) and heart rate (HR) responses to the Valsalva maneuver.

In a patient with sympathetic neurocirculatory failure, during Phase II the blood vessels fail to constrict reflexively, and so blood pressure falls progressively and does not increase toward baseline at the end of Phase II.



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

During Phase IV, because of the lack of tightening of blood vessels, there is no rapid increase in blood pressure and no Phase IV overshoot of pressure. Instead, the blood pressure gradually increases slowly back to the baseline value.

The responses of pulse rate to the *Valsalva maneuver* depend mainly on changes in *parasympathetic nervous system* outflow to the heart via the *vagus* nerve. In Phase II, the pulse rate increases, and in Phase IV the pulse rate returns rapidly to baseline. In *parasympathetic neurocirculatory failure* or *baroreflex failure,* the pulse rate remains unchanged both during and after performance of the maneuver.

Note that one must monitor the beat-to-beat blood pressure changes 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.*

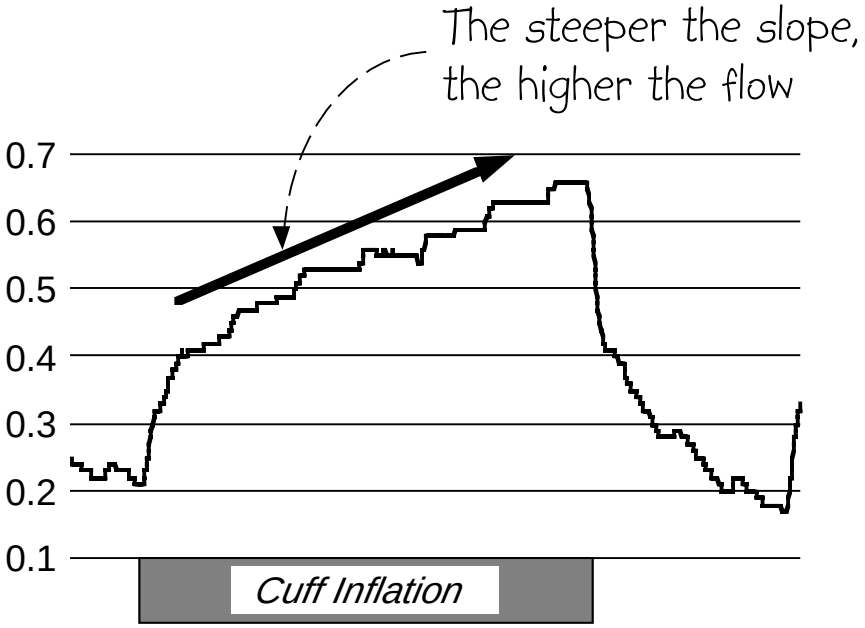
The recent introduction of special testing devices has provided non-invasive means to follow blood pressure beat-to-beat and detection of *sympathetic neurocirculatory failure.*

Forearm Blood Flow

This non-invasive test measures the rate of blood flow in the forearm. From the *forearm blood flow (FBF)* and the blood pressure *(mean arterial pressure, MAP),* the *forearm vascular resistance (FVR)* can be estimated. In the garden hose analogy, the *FVR* would correspond to the extent of tightening of the nozzle. One of the main ways the body has to regulate blood pressure is by regulating *vascular resistance,* using the *sympathetic nervous 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 nervous system outflows, which increases *peripheral resistance* to blood flow and helps keep the average *blood pressure (mean arterial pressure)* normal, despite the decrease in *cardiac output.*

To measure *forearm blood flow,* 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 to just above the *venous pressure* but below the *arterial pressure.*



Resistance = Pressure / Flow

FVR = MAP / FBF

TPR = MAP / CO

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

This is like tightening a tourniquet around the upper arm, for obtaining 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 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 is high, then the volume of the forearm increases rapidly after the cuff is inflated; and if the rate of blood flow is low, then the volume of the forearm increases more slowly. By a simple calculation we 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)* can be estimated 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)*. When a person stands up or is tilted on a tilt-table as part of *tilt-table testing*, the *forearm vascular resistance* normally increases. A failure of the *forearm vascular resistance* to increase during standing is a sign of *sympathetic neurocirculatory failure.*

Incidentally, the logo of the National Dysautonomia Research Foundation is based on the signal used to

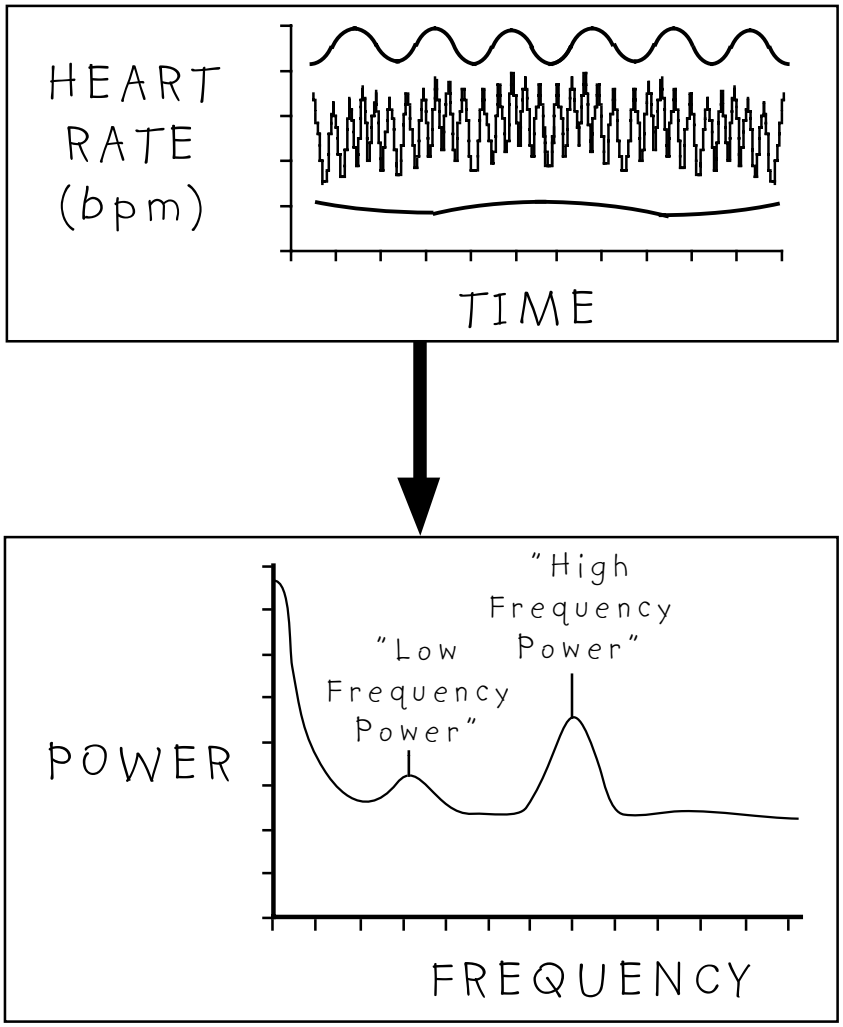
measure *forearm vascular resistance.* Just before a patient faints, the *forearm blood flow* increases. The logo shows the increase in the *forearm blood flow* on repeated measurements, as in the background the patient's skin color turns from a healthy red color to a deathly blue!

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.

The change in pulse rate from breathing is sometimes called *respiratory sinus arrhythmia.* This sounds like an abnormal heart rhythm, but it actually is a sign of a healthy heart. *Respiratory sinus arrhythmia* is thought to result from changes in *parasympathetic nervous system* influences on the heart.

If one graphs the size of the oscillation as a function of the frequency of the heartbeats, then at the frequency of breathing, there is 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. This sort of analysis has revealed a second peak of *power,* at a lower frequency than the frequency of breathing.



Power spectral analysis is a non-invasive way to interpret heart rate changes.

Researchers have thought that this low frequency *power* is related to *sympathetic nervous system* influences on the heart.

Power spectral analysis of heart rate offers the advantages of being safe, technically easy, and fast. The main disadvantage is that the meaning of *low frequency power* as an index of *sympathetic nervous system* activity in the heart remains in dispute.

Tilt-Table Testing

Tilt-table testing is done to see if standing up *(orthostasis)* provokes a sudden fall in blood pressure *(neurally mediated hypotension)*, an excessive increase in pulse rate *(postural tachycardia syndrome, POTS)*, or fainting *(neurally mediated syncope)*.

Tilt-table testing is used to detect POTS or neurocardiogenic 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) up to about 45 minutes. If the patient tolerates the tilting for this period,

then the patient may receive a drug, such as *isoproterenol* or *nitroglycerine,* which might provoke a sudden fall in blood pressure or loss of consciousness. As soon as the test becomes positive, the patient is put back into a position lying flat or with the head down, and sometimes fluid is given by vein. Patients usually recover consciousness within a minute or two.

Tilt-table testing is a form of *provocative* test. The doctors are hoping to reproduce the patient's problem in a controlled laboratory situation. The testing is quite safe when done by experienced personnel, in a setting where emergency backup is available.

There are several disadvantages of *tilt-table testing.* One is the issue of *false-positive* results, especially when a drug such as *isoproterenol* 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."

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 or nothing to suggest treatments that might be effective.

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 beginning as soon as the tilting starts.

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 nervous system* traffic to sweat glands in the skin. The chemical messenger, *acetylcholine*, is released, and the *acetylcholine* acts on the sweat glands to stimulate production of sweat.

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

There are several ways to measure *sympathetic cholinergic* sweating in response to external heat *(thermoregulatory sweat test, TST).* One is from sprinkling starch with iodine all over the body. When the starch-iodine combination is wetted, the powder turns brown. One can then photograph the body and see which parts sweated. Sometimes other powder-dye combinations are used. When the skin becomes sweaty, the ability to conduct electricity increases dramatically, 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 attached to the skin.

Another way to test sweating is from the *galvanic skin response (GSR)* or *skin sympathetic test (SST).* The *galvanic skin response* is part of polygraphic "lie detector" testing. When a person is suddenly distressed, or a small electric shock is delivered, increased activities of the *sympathetic nervous system* and the a*drenomedullary hormonal system* evoke sweating. One can also measure sweating from humidity in a capsule applied to the skin.

Advantages of sweat tests are that they are generally safe, simple, and quick. A disadvantage is that they only measure *physiological* changes as a result of release of *acetylcholine* from *sympathetic nerve terminals* or *epinephrine* from the *adrenal gland.* There are *dysautonomias* where the patient has normal sweating.

Another disadvantage is that sweat tests are only indirectly and complexly related to activity of the *sympathetic nervous system,* and they provide little information about the exact mechanism of the *dysautonomia.*

The Cold Pressor Test

In the *cold pressor test,* the patient dunks a hand into ice-cold water. This rapidly increases the blood pressure, by increasing activity of the *sympathetic nervous system.* Since the test involves not only cold but also pain, the *cold pressor test* can only be done for a minute or two. A similar limitation applies for isometric handgrip exercise.

Neuropharmacologic Tests

QSART

"QSART" stands for *"Quantitative Sudomotor Axon Reflex Test."*

The QSART is a special form of sweat 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 nerve terminals* in the heart and blood vessel walls. The *QSART* is a test of the ability of *sympathetic nerve terminals* in the skin to release *acetylcholine* and increase sweat production.

As in some other sweat tests, in the *QSART* procedure, dried nitrogen (or dried air, or air with a known amount of humidity) is pumped at a controlled rate through a small plastic, dome-like capsule placed on the skin. When the person sweats, the humidity in the chamber increases, as the sweat droplets evaporate. This provides a rapid measure of sweat production. For *QSART* testing,

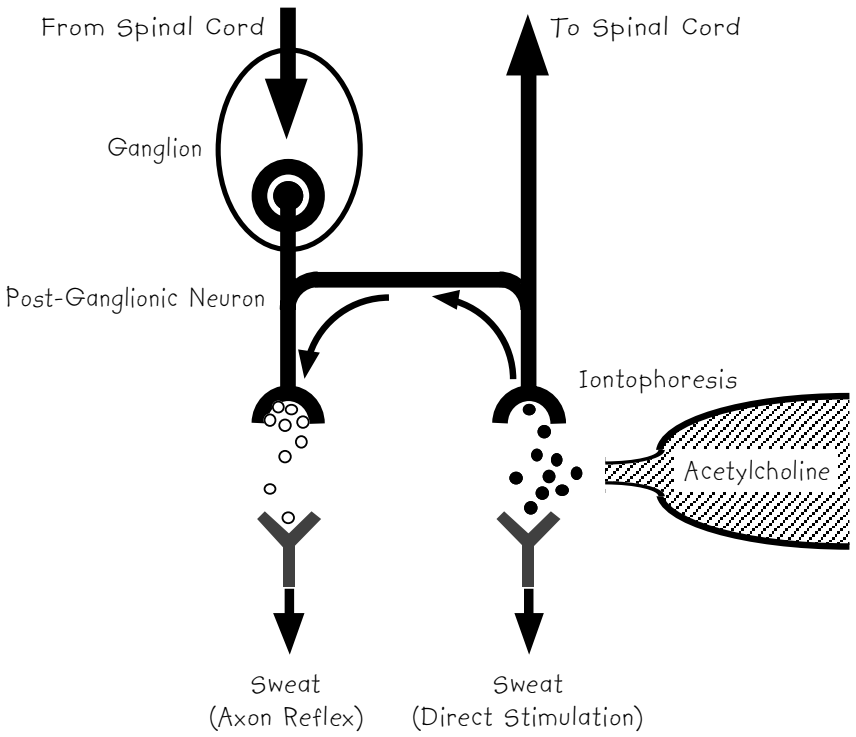


Diagram of the QSART test.

a drug that stimulates *acetylcholine receptors* (for instance *acetylcholine* itself) 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 nerve terminals* under the nearby plastic capsule release the body's own

acetylcholine, resulting in sweat production measured by increased humidity in the capsule.

If a person had a loss of *sympathetic nerve terminals* that release *acetylcholine* (loss of *cholinergic* terminals), 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. On the other hand, if the person had intact *sympathetic cholinergic nerve terminals,* then applying acetylcholine to a patch of skin near the test capsule would increase the humidity in the capsule. If the person had a brain disease that prevented increases in *sympathetic 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 failure* due to loss of *cholinergic terminals* from failure due to abnormal regulation of *sympathetic nerve traffic* to intact *cholinergic terminals.*

Advantages of the *QSART* are that it is generally safe, quick, quantitative, and easy for a technician to perform. There are also several disadvantages. The equipment required is fairly expensive, and relatively few centers have *QSART* testing available, so that **availability** of the test is an issue. As in other tests where the key factor being measured is *physiological* (in this case, sweat

production), the results are **indirect**. For instance, a problem with the ability to make *acetylcholine* in the nerve terminals or with the ability of acetylcholine to bind to its *receptors* in the sweat glands would result in the same abnormal *QSART* responses as if the *sympathetic cholinergic terminals* were lost. Finally, *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**.

Trimethaphan Infusion Test

Trimethaphan is a type of drug called a *ganglion blocker*.

In the *autonomic nervous system,* control signals from the brain and spinal cord are relayed through the *ganglia,* and nerves from the *ganglia, postganglionic* nerves, deliver those signals to the *nerve terminals* near or in the target tissues. The control signals are relayed in the *ganglia* by release of the chemical messenger, *acetylcholine,* which binds to specific receptors on the *postganglionic* cells, called *nicotinic receptors.*

Stimulation of the *nicotinic receptors,* such as by *nicotine* itself, *increases postganglionic nerve traffic* in both the *parasympathetic nervous system* and the *sympathetic nervous system.*

Trimethaphan does just the opposite. It blocks *nicotinic receptors* in the *ganglia*. By blocking the *receptors*,

trimethaphan blocks the transmission of nerve impulses in the *ganglia* to the *postganglionic nerves* of the *sympathetic nervous system* and *parasympathetic nervous system.* The rates of *sympathetic nerve traffic* and *parasympathetic nerve traffic* fall to virtually zero.

Because of the blockade of transmission of nerve impulses in *ganglia, trimethaphan* normally produces 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 nerve traffic. Trimethaphan* therefore always produces a fall in blood pressure when the person stands up, called *orthostatic hypotension.* If the person is lying down at the time, then *trimethaphan* produces a relatively small decrease in blood pressure. Probably the most noticeable effect of *trimethaphan* 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 *trimethaphan infusion test,* the 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 norepinephrine levels* or levels of other neurochemicals.

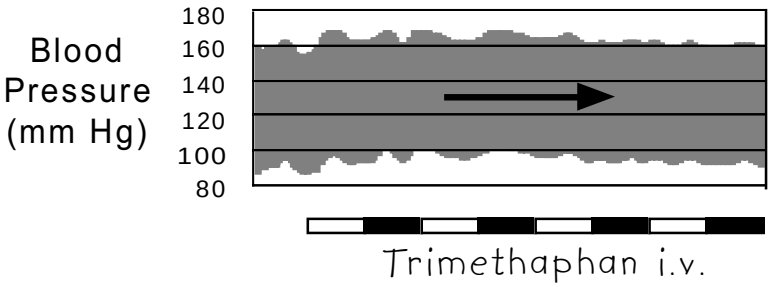
If a patient had *autonomic failure* due to a loss of *sympathetic nerve terminals,* such as in *Parkinson's*

disease with orthostatic hypotension, there would be no release of *norepinephrine* from the nerve terminals, because of the absence of the terminals. *Trimethaphan* in such a patient would not affect the blood pressure. But if a patient had *autonomic failure* due to a brain disease, such as the *Shy-Drager syndrome (multiple system atrophy with sympathetic neurocirculatory failure)*, where there was an inability to regulate *sympathetic nerve traffic* to intact terminals, there might be ongoing, unregulated release of *norepinephrine* from the nerve terminals. *Trimethaphan* in such a patient would decrease the blood pressure.

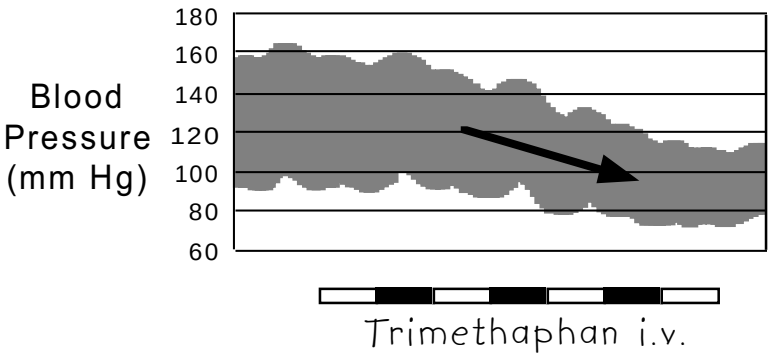
The *trimethaphan infusion test* therefore can provide information about whether *autonomic failure* is associated with a loss of *sympathetic nerve terminals* or from failure of the brain to regulate *sympathetic nerve traffic* appropriately.

In some patients with long-term high blood pressure *(hypertension)*, the *hypertension* seems to reflect 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

Parkinson's Disease with Orthostatic Hypotension



Shy-Drager Syndrome



The trimethaphan infusion test can help to identify different causes of autonomic failure.

heart *(cardiac output)* and tightening of blood vessels *(vasoconstriction).* By either or both mechanisms, the blood pressure would be 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* would be expected to decrease the rate of *norepinephrine* release from the *sympathetic nerve terminals,* 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 nervous system, trimethaphan* would not be expected to decrease the blood pressure as much.

Because *trimethaphan* is a potent blocker of the *sympathetic nervous system* and the *parasympathetic nervous system,* the drug must be given at a carefully controlled rate, by personnel who are well acquainted with its effects. If the dose is too high, then the blood pressure (especially the *systolic* blood pressure) can fall too much. The effects of *trimethaphan* wear off quickly after the infusion is stopped, and so if too much drug is being given, decreasing the infusion rate or stopping the infusion will eliminate the side effects within minutes. An antidote drug should be available that directly stimulates *norepinephrine receptors.* Sometimes *trimethaphan* can evoke release of *histamine,* which can produce itching, wheezing, or decreased blood blood pressure. An anti-histamine drug should also be available.

Yohimbine Challenge Test

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 nerve terminals,* and in blood vessel walls.

When *alpha-2 adrenoceptors* in the brain are blocked, this increases *sympathetic nerve traffic. Alpha-2 adrenoceptors* on *sympathetic nerve terminals* act like a brake on *norepinephrine* release from the terminals. When *alpha-2 adrenoceptors* on *sympathetic nerve terminals* are blocked, this increases the amount of *norepinephrine* release for a given amount of *sympathetic nerve traffic.* Yohimbine, by blocking *alpha-2 adrenoceptors* in the brain and on *sympathetic nerve terminals,* therefore releases *norepinephrine* from the terminals. The released norepinephrine binds to *alpha-1 adrenoceptors* in blood vessel walls, causing an increase in blood pressure.

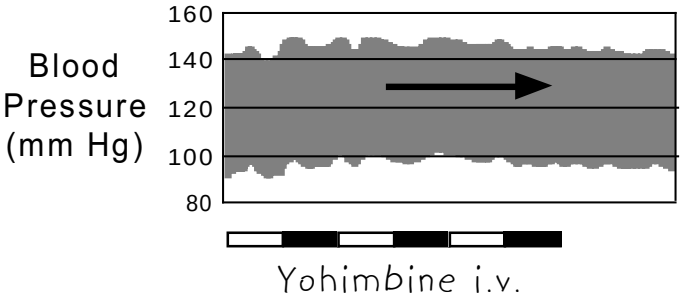
Because of the blockade of *alpha-2 adrenoceptors* in the brain, *yohimbine* can produce any of several **behavioral or emotional effects,** which vary from person to person. *Yohimbine* can cause an increase in alertness or feelings such as anxiety or sadness, or, on the other hand, happiness or a sense of energy. Rarely, yohimbine can cause a panic attack.

Yohimbine usually causes *trembling,*which sometimes is so severe that the teeth chatter, and it sometimes also causes **paleness** of the skin, **goosebumps**, and **hair standing out,** as if the person were either very cold or distressed. Actually, the body temperature does not fall at all, and the person does not feel cold. Another sometimes noticeable effect of *yohimbine* is an **increase in salivation.** This is probably because of an increase in the rate of *sympathetic nerve traffic* to the *salivary glands.*

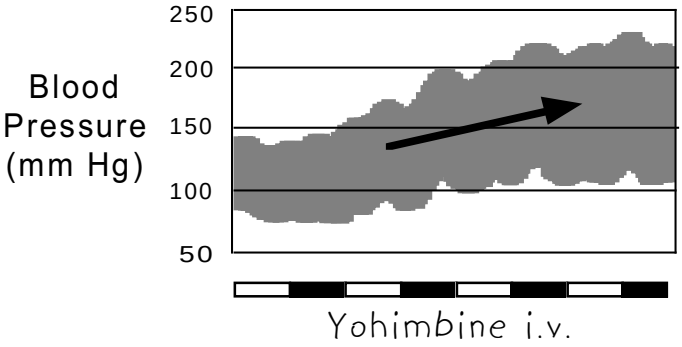
In the *yohimbine challenge test,* the drug is given by vein for several minutes or given by mouth as a single dose. *Yohimbine* given by vein is currently an investigational drug. The blood pressure and pulse rate are monitored frequently or continuously, and blood often is sampled from an indwelling catheter in an arm vein, for measurements of *plasma norepinephrine levels* or levels of other neurochemicals.

If a patient had *autonomic failure* due to a loss of *sympathetic nerve terminals,* such as in *Parkinson's disease with orthostatic hypotension,* there would be no release of *norepinephrine* from the nerve terminals, regardless of the nerve traffic, because of the absence of the terminals. *Yohimbine* in such a patient would not affect the blood pressure. But if a patient had *autonomic failure* due to a brain disease, such as the *Shy-Drager syndrome (multiple system atrophy with sympathetic neurocirculatory failure),* where there was an inability to regulate *sympathetic nerve traffic* to intact terminals, *yohimbine* would increase the blood pressure, and

Parkinson's Disease with Orthostatic Hypotension



Shy-Drager Syndrome



The yohimbine challenge test can help to identify different causes of autonomic failure.

because of the inability to regulate *sympathetic nerve traffic*, the brain would not reflexively decrease the *sympathetic nerve traffic* to compensate for the increased blood pressure. This means that infusion of yohimbine into such a patient might produce a large increase in blood pressure. If the patient already had high blood pressure, or if the doctor already strongly suspected a disease such as the *Shy-Drager syndrome,* then the dose would be decreased, or the doctor might decide that carrying out the test would not be worth the risk.

In some patients with long-term high blood pressure *(hypertension),* the *hypertension* seems to reflect 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 patients with *hypernoradrenergic hypertension,* some of the released *norepinephrine* binds to the *alpha-2 adrenoceptors* on the *sympathetic nerve terminals,* and this puts a brake on the *norepinephrine* release. Infusion of *yohimbine* by vein into such patients increases both blood pressure and the *plasma norepinephrine level,* by blocking this restraint. The finding of a large increase in blood pressure coupled with a large increase in the

plasma norepinephrine level provides support for the 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 nerve terminals,* the released *norepinephrine* is not inactivated by "recycling" 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* therefore produces a large increase in the *plasma norepinephrine level* and large increases in the pulse rate and blood pressure. *Yohimbine* can also evoke *panic* or chest pain or pressure that can mimic the chest pain or pressure in *coronary artery disease.*

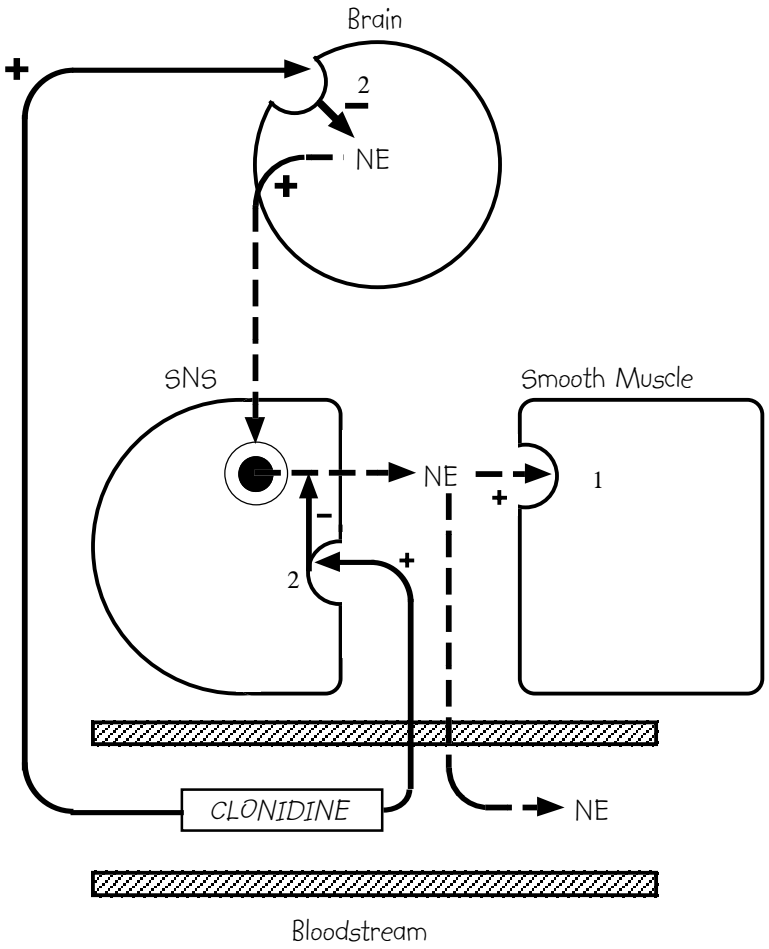
The *yohimbine challenge test* therefore can provide information about whether *autonomic failure* is associated with a loss of *sympathetic nerve terminals* or from failure of the brain to regulate *sympathetic nerve traffic* appropriately. The test can also be used to identify *hypernoradrenergic hypertension* or *NET deficiency.* The effects of *yohimbine* wear off over several minutes, once the infusion ends. If the blood pressure increase were excessive, the quickest way to bring the pressure down would be to stop the infusion and, if the patient had *orthostatic hypotension,* have the patient stand up. Very rarely, an antidote drug that stimulates *alpha-2 adrenoceptors*, such as *clonidine,* has to be given.

Because *yohimbine* given by vein is an investigational drug that produces important effects on functions of the *sympathetic nervous system*, the *yohimbine challenge test* should be done only by personnel who are well acquainted with its effects.

Clonidine Suppression Test

Clonidine stimulates *alpha-2 adrenoceptors* in the brain, on *sympathetic nerve terminals,* and in blood vessel walls. When *alpha-2 adrenoceptors* in the brain are stimulated, this decreases *sympathetic nerve traffic, and* when *alpha-2 adrenoceptors* on *sympathetic nerve terminals* are stimulated, this decreases the amount of *norepinephrine* release for a given amount of *sympathetic nerve traffic.*

Released *norepinephrine* binds to both *alpha-2 adrenoceptors* and *alpha-1 adrenoceptors* in blood vessel walls. Even though *clonidine* stimulates *alpha-2 adrenoceptors*, which would constrict blood vessels, the drug is so powerful in decreasing release of *norepinephrine* that normally after a dose of *clonidine* the blood pressure falls. *Clonidine* is an approved drug for the treatment of long-term high blood pressure *(hypertension).*



The clonidine suppression test can be helpful in identifying causes of abnormal levels of norepinephrine (NE), the chemical messenger of the sympathetic nervous system (SNS).

By stimulating *alpha-2 adrenoceptors* in the brain, *clonidine* usually produces some sedation. It can cause a decrease in alertness or a decrease in the sense of energy. *Clonidine* is effective in relieving symptoms of withdrawal from alcohol or opiate-type narcotics.

Clonidine can produce a dry mouth and a small decrease in the pulse rate. This is probably because of a decrease in the rate of *sympathetic nerve traffic* to the *salivary glands* and heart.

In the *clonidine suppression test*, the drug is given by mouth, usually at a dose of 300 micrograms, which would be the total amount of the drug given in divided doses in a day. The blood pressure and pulse rate are monitored over the course of a few hours, and blood is sampled from an indwelling catheter in an arm vein, for measurements of *plasma norepinephrine levels*.

If a patient had *autonomic failure* due to a loss of *sympathetic nerve terminals,* such as in *Parkinson's disease with orthostatic hypotension*, there would be no release of *norepinephrine* from the nerve terminals, regardless of the nerve traffic, because of the absence of the terminals. *Clonidine* in such a patient would not affect the blood pressure, or it might actually increase the blood pressure, due to stimulation of *alpha-2 adrenoceptors* in the blood vessel walls.
But if a patient had *autonomic failure* due to a brain disease, such as the *Shy-Drager syndrome (multiple system atrophy with sympathetic neurocirculatory failure),* where there was an inability to regulate

sympathetic nerve traffic to intact terminals, there would be *norepinephrine* in the terminals, and *clonidine* would inhibit its release. *Clonidine* in such a patient would decrease the blood pressure, and because of the inability to regulate *sympathetic nerve traffic*, the brain would not reflexively increase the *sympathetic nerve traffic* to compensate for the decreased blood pressure. This means that *clonidine* might produce a large decrease in blood pressure.

In some patients with long-term high blood pressure *(hypertension)*, the *hypertension* is associated with an overall increase in the rate of nerve traffic in the *sympathetic nervous system.* The blood pressure would be high because of the high rate of delivery of *norepinephrine* to its receptors—*hypernoradrenergic hypertension. Clonidine* binds to the *alpha-2 adrenoceptors* on the *sympathetic nerve terminals,* and this puts a brake on the *norepinephrine* release. *Clonidine* given to such patients decreases both blood pressure and the *plasma norepinephrine level.*The finding of a large decrease in blood pressure coupled with a large decrease in the *plasma norepinephrine level* provides support for the diagnosis of *hypernoradrenergic hypertension.*

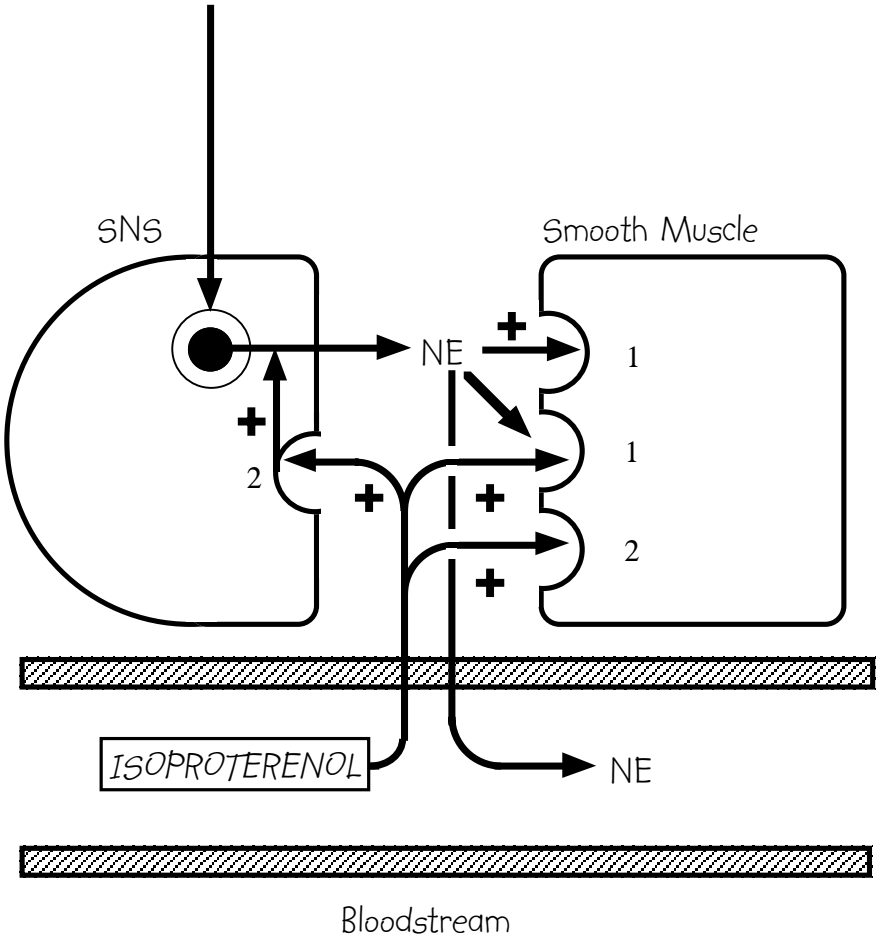
Rarely, *hypernoradrenergic hypertension* results from a tumor that produces *catecholamines* such as *norepinephrine* and *epinephrine.* The tumor is called a *pheochromocytoma.* The *clonidine suppression test* is an accepted diagnostic test for *pheochromocytoma.* If the *hypernoradrenergic hypertension* resulted from a high rate of *sympathetic nerve traffic,* then *clonidine* would

decrease the elevated *plasma norepinephrine level.* But if the patient had a “pheo,” which would produce *catecholamines* independently of the rate of *sympathetic nerve traffic, clonidine* would fail to decrease the *plasma norepinephrine level.* In other words, in a positive *clonidine suppression test* for *pheochromocytoma,* the *plasma norepinephrine level* fails to decrease after a dose of *clonidine,* despite the presence of *hypernoradrenergic hypertension*.

The effects of *clonidine* wear off over several hours. Patients can feel sedated even up to the next day. Therefore, patients should not drive or operate heavy machinery for at least 24 hours after having a *clonidine suppression test.* The drug rarely if ever produces a dangerous fall in blood pressure.

Isoproterenol Infusion Test

Isoproterenol (brand name Isuprel™) stimulates *beta-adrenoceptors. Beta-adrenoceptor* stimulation has several important effects in the body. Stimulation of *beta-adrenoceptors* in the heart increases the rate and force of the heartbeat and therefore 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



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

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, decreasing the resistance to blood flow in the body as a whole *(total peripheral resistance).* Stimulation of *beta-adrenoceptors* on *sympathetic nerve terminals* increases the release of *norepinephrine.*

Isoproterenol is infused by vein as part of diagnostic testing for a few types of dysautonomias, which appear to overlap and may be different forms of the same problem. In the *hyperdynamic circulation syndrome,* the patient has a relatively fast pulse rate, high *cardiac output,* a variable blood pressure that tends to be increased, a tendency towards panic or anxiety attacks, excessive increases in pulse rate in response to *isoproterenol* given by vein, and improvement by treatment with the *beta-adrenoceptor blocker, propranolol.* The same holds true for many relatively young patients with early, borderline *hypertension.* Patients with the *postural tachycardia syndrome (POTS)* also can have a fast pulse rate, even when lying down, excessive increases in pulse rate during *isoproterenol* infusion, and sometimes panic evoked by the infusion.

Isoproterenol infusion is also done as part of tilt-table testing in patients with *chronic fatigue syndrome* or *chronic orthostatic intolerance.* After prolonged 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 might arise from stimulation of the heart by *isoproterenol* or from relaxation of blood vessels in skeletal muscle, which would shunt blood away from the brain and towards the limbs.

Of course, this brings up the issue of how frequently a healthy person might have one of these reactions in response to *isoproterenol* infusion in the setting of prolonged tilting, which would be a false-positive result.

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. The risk of these side effects disappears as soon as the drug wears off. Because of the increase in pulse rate and the force of the heartbeat, *isoproterenol* infusion increases the work of the heart, and this might cause problems such as chest pressure in patients with *coronary artery disease.*

Neurochemical Tests

Neurochemical tests of *autonomic nervous system* function mainly involve the *sympathetic nervous system* or *adrenomedullary hormonal system.* This is because the main chemical messengers of these systems, *norepinephrine* and *epinephrine (adrenaline),* are relatively stable and can be measured in the plasma, while the main chemical messenger of the *parasympathetic nervous system*, *acetylcholine*, undergoes rapid breakdown and cannot be measured in the plasma.

Plasma Norepinephrine Levels

Since *norepinephrine* is the main chemical messenger of the *sympathetic nervous system*, doctors have often used the *plasma norepinephrine level* as an index of *sympathetic nervous system* "activity" in the body as a whole.

Plasma norepinephrine is used to test the sympathetic nervous system.

There is some validity in doing this, but 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 therefore 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 nerve terminals* actually makes its way into the bloodstream. Most is "recycled" back into the nerve terminals, by a process called *"Uptake-1,"* using a special transporter called 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. It happens that *norepinephrine* is cleared from the plasma extremely rapidly. This means

that a person might have a high *plasma norepinephrine level* because of a problem with the ability to remove *norepinephrine* from the plasma, such as in kidney failure.

Third, *norepinephrine* is produced in *sympathetic nerve terminals* by the action of three enzymes (*tyrosine hydroxylase*, or *TH, DOPA decarboxylase*, or *DDC,* and *dopamine-beta-hydroxylase*, or *DBH),* 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 nerve terminals.* 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 low *plasma norepinephrine levels,* regardless of the rate of *sympathetic nerve traffic.*

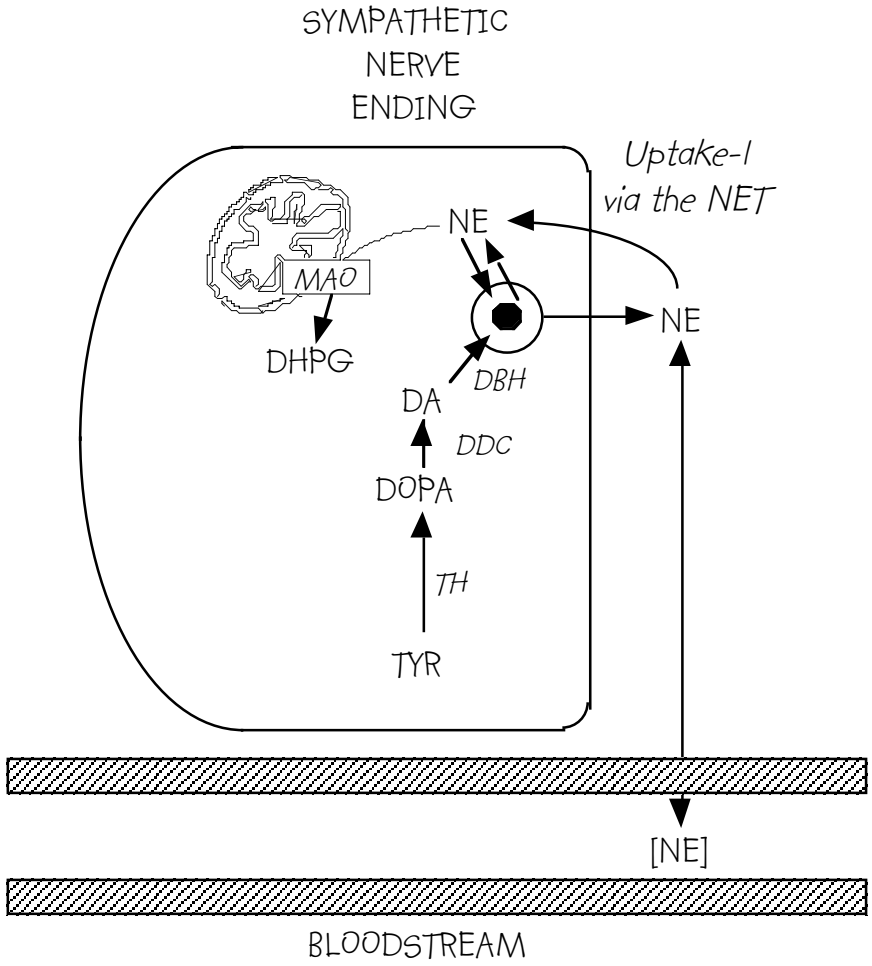
Fourth, the *plasma norepinephrine level* usually is measured in a blood sample drawn from a vein in the arm. Because the skin and skeletal muscle in the forearm and hand contain many *sympathetic nerve terminals,* the *plasma norepinephrine level* in blood from an arm vein is determined not only by the amount of *norepinephrine* release from *sympathetic nerve terminals* 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 doubles within 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.

Plasma Epinephrine (Adrenaline) Levels

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

Plasma epinephrine (adrenaline) is used to test the adrenomedullary hormonal system.



Several factors influence plasma norepinephrine levels.

Nevertheless, some of the same factors that make interpreting *plasma norepinephrine levels* difficult can complicate interpreting *plasma epinephrine levels.* A large number of common and difficult to control life experiences that influence activity of the *adrenomedullary hormonal 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. *Epinephrine* is a very powerful *hormone.* Not surprisingly, the *plasma epinephrine level* normally is very low. The level can be so low that it is about at or below the limit of sensitivity of the measurement technique, which would invalidate the result. Other chemicals besides *epinephrine* 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 *epinephrine* in some assay procedures.
Because of these issues, it is important that blood sampling and chemical assays for *plasma epinephrine levels* be carried out by experienced and expert personnel.

Neuroimaging Tests

Compared to other types of testing for *dysautonomias,* testing using *neuroimaging* is new.

Neuroimaging is a way to actually see nervous system tissue, such as in the brain. In testing for *dysautonomias,* the neuroimaging involves seeing the *sympathetic nerves* in an organ outside the brain, such as in the heart.

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 because 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 vein, and they are delivered to the heart muscle by way of the *coronary arteries.* This means that one must be able to distinguish a local loss of radioactivity in the scan that is due to loss of *sympathetic nerves* from a local loss that is due to a place where the *coronary artery* is blocked, because

either nerve loss or coronary blockage could 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

In the United States, *sympathetic neuroimaging* is available in few centers but is available fairly widely in European countries and Japan. Worldwide, probably the most commonly used *sympathetic neuroimaging* agent is *^{123}I-metaiodobenzylguanidine*, or *^{123}I-MIBG*. *^{123}I-MIBG* is a radioactive form of a drug that is taken up by sympathetic nerve terminals, making them visible on a nuclear medicine scanner.

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.* This is a radioactive form of the *catecholamine, dopamine.* After injection of *6-*

[^{18}F]fluorodopamine, by vein, the drug is taken up by sympathetic nerve terminals, 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 imagine that you had many little Geiger counters all around the box. Each counter would detect a different amount of radioactivity, depending on the shape of the object and the distance of the counter from the object. If you had a way to construct a picture, such as in a newspaper photo, where the size and intensity of each dot depended on the amount of radioactivity, then you could construct an image of the object inside 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.

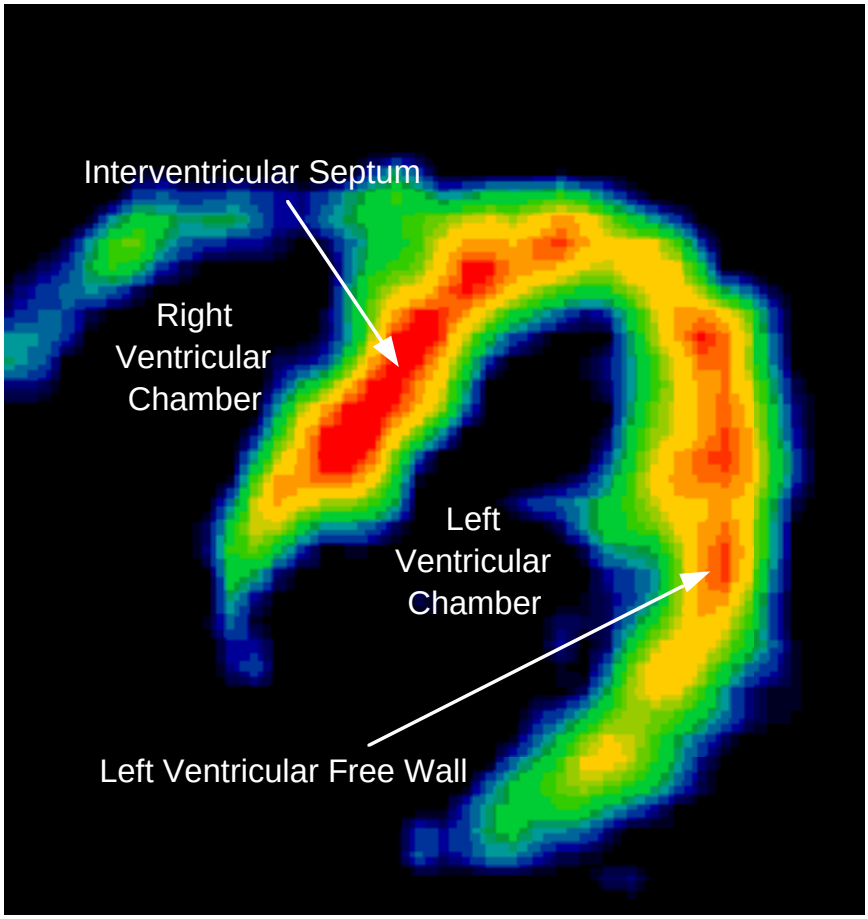
A *positron* emitter is a type of radioactive substance that releases a short-lived form of radiation that can penetrate the body and reach detectors outside it, enabling construction of a *PET* scan. Other scans in nuclear medicine use a somewhat different source of radioactivity, but the idea is about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

Fluorodopamine is structurally similar to the biochemicals of the *sympathetic nervous system,*

norepinephrine (noradrenaline) and *epinephrine (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 nerve endings*, 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 nerve endings* there. Because there are so many sympathetic nerve endings 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 nerve terminals, such as in *pure autonomic failure* and in *Parkinson's disease,* because even when the blood flow to the heart muscle is normal, there is no heart visible in the *PET scan!*

A much more difficult issue is whether analysis of the amount of radioactivity in the heart can provide information about how the *sympathetic nerve terminals* are functioning. This is a matter of research interest now.



Fluorodopamine PET scanning can show the sympathetic nerves in the heart muscle.