Investigating autonomic control of the cardiovascular system: a battery of simple tests

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The autonomic nervous system has a profound control over the cardiovascular system. Heart rate (HR) is under the dual control of sympathetic and parasympathetic divisions, and the resultant HR reflects the balance of these opposing influences. The contractility, and therefore stroke volume, of the heart is influenced predominantly by the sympathetic division, directly via sympathetic innervation of cardiac muscle, and indirectly by release of catecholamines by the adrenal medulla. Vasoconstrictor tone is influenced principally by sympathetic nerves, along with medullary release of catecholamines. Although parasympathetic nerves have a major influence in some blood vessels (necessary for the propagation of our species, for example), this innervation has little or no direct effect on the maintenance of arterial blood pressure (BP). The objective of this set of teaching laboratory activities is to produce normal physiological disturbances in a way that the cardiovascular consequences can be easily predicted and measured. This serves to demonstrate how autonomic control of the cardiovascular system normalizes cardiac output and BP in response to these challenges. These tests are also used clinically to help investigate potential damage to this control. In addition to demonstrating basic physiological principals that can be applied to pathological conditions, these laboratory activities involve simple measurements of arterial BP along with the calculation of HR from R-R intervals on ECG records, instilling the first principles of HR measurement that may not otherwise be appreciated by students using automated equipment. They nurture further skills of data manipulation, group dynamics, and an appreciation of normal variability.

Background

The autonomic nervous system exerts a profound influence over many body systems and includes control of the cardiovascular system, which frequently deals with challenges to systemic BP on a second-to-second basis. This involves elements of the classic reflex arc: receptors that generate afferent sensory information (in this case, primarily from the arterial baroreceptors) as well as central processing and integration (within medullary regions of the brain stem), which then produce activity in efferent autonomic nerves to the effector organs of the heart and blood vessels (see Refs. 7 and 16). These elements are essential for adequate BP control yet are vulnerable to disease processes that are currently on the increase worldwide, such as diabetic autonomic neuropathy (6, 17). With diabetic autonomic neuropathy, damage to the innervation of the heart (particularly the sympathetic division) reduces the heart’s capacity to increase HR and stroke volume with normal, physiological reductions of BP. Similarly, damage of sympathetic innervation to blood vessels may reduce their constrictive capacity and their ability to increase peripheral resistance. These factors contribute to common cardiovascular complications such as orthostatic hypertension and poor circulation to the extremities, including the skin and feet (10). Damage to these thin, unmyelinated nerves is common to several types of neuropathy (1, 6, 10, 12). By measuring HR and BP, the activities outlined here provide insights into these control mechanisms at work during various cardiovascular challenges and, in the clinical setting, are used diagnostically to assess for deficits in autonomic reflex control of the cardiovascular system. Individual clinicians use different combinations of procedures that often include those outlined here to develop an overall picture of the autonomic control of the cardiovascular system, among other tests of autonomic function, to aid in the diagnosis of nervous system deficits (10, 12).

Learning Objectives

After completing this activity, the student will be able to:
1. Describe easily measurable and accessible aspects of cardiovascular control
2. Discuss the considerations of standardizing experimental protocols to allow valid comparison of results between subjects
3. Conduct the following three cardiovascular challenges while gathering HR and/or BP data: deep breathing, the Valsalva maneuver, and standing from lying down

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4. Describe and explain the autonomic contributions to cardiovascular consequences of three cardiovascular challenges.

5. Relate these findings to results that may be expected from subjects with forms of autonomic neuropathy.

**Activity Level**

This activity is suitable for basic medical science and medical students who are studying control of the cardiovascular system and/or the autonomic nervous system.

**Prerequisite Student Knowledge or Skills**

Before doing this activity, students should have a basic understanding of:

1. The autonomic nervous system, in particular, sympathetic and parasympathetic innervation of the heart and sympathetic innervation of blood vessels

2. Physiological processes contributing to the control of HR and BP

Students should know how to:

1. Measure HR from a standard ECG

2. Measure arterial BP with a sphygmomanometer/BP cuff

**Time Required**

The procedures and analysis themselves take <90 min, although discussion beforehand about the parameters that can be measured and how to standardize them may add 30 min.

**METHODS**

**Equipment and Supplies**

The following equipment and supplies are needed per group (suggestion of 3 students/group):

1. An ECG recording device, ideally from lead II, to allow HR measurements

2. For BP measurements, a sphygmomanometer/BP cuff and stethoscope

3. For the Valsalva maneuver: a modified sphygmomanometer (we use a Riester ri-sani) with a tube (~15 cm) attached to one end of a 20-ml syringe and the other end (with flanges trimmed) attached to a rubber snorkeling mouthpiece. The dial has a mark made visible at 40 mmHg.

4. A stopwatch/clock

5. A suitable surface for the student to lie down. We use clean laboratory benches with a one blanket laid out per subject.

**Human Subjects Approval**

These noninvasive experiments do not require ethical approval at our institution. Adopters of these activities are responsible for obtaining permission for human research from their home institution. For a summary of the "Guiding Principles for Research Involving Animals and Human Beings," please see http://www.the-aps.org/mm/Publications/Ethical-Policies/Animal-and-Human-Research.

**Instructions**

Experiments 1–3 are based on the changes in HR before and after the performance of a particular procedure by the subject. Usually the necessary measurements are made on lead II of the ECG. It is important to obtain a short strip of lead II while the subject lies quietly at rest and then continue recording as each test is carried out.

*Experiments 3 and 4 should be combined with one observer recording the ECG and the other observer making the BP measurements.*

**Experiment 1: the HR response to deep breathing.** The subject lies quietly and, when instructed, breathes at 6 breaths/min for 1 min (5 s in, 5 s out). Mark the onset of each inspiration and each expiration on the recording paper. Measure the shortest R-R interval after each inspiration and the longest R-R interval after each expiration. Calculate the corresponding maximum HR after each inspiration and the minimum HR after each expiration. Calculate the inspiratory/expiratory difference for each of the six cycles, as follows:

\[
HR = \frac{60 \times 25}{d}
\]

where \(d\) is the R-R interval (in mm) (paper speed = 25 mm/s).

Calculate the mean difference (inspiratory HR − expiratory HR) in beats per minute.

**Experiment 2: the HR response to a Valsalva maneuver.** Record the resting strip with the subject breathing quietly through the mouthpiece to the atmosphere. Continue recording as he/she takes a breath in and then blows out through the mouthpiece into a manometer and holds it at a pressure of 40 mmHg for 15 s. Continue recording for 30 s after the release of the strain. Mark the inspiration and the start and end of the strain period on the recording paper.

The result is expressed as the Valsalva ratio, as follows:

\[
\text{Valsalva ratio} = \frac{\text{Longest R-R interval after the release of the strain}}{\text{Shortest R-R interval during the strain}}
\]

**Experiment 3: the HR response to standing.** For students who may be relatively inexperienced in taking ECG recordings and BP measurements, it is better to carry out experiments 3 and 4 as separate procedures to optimize the accuracy of measurements. After basal ECG recording, the subject is asked to stand unsupported, and recording is continued through this and for 1 min after standing. Mark the start to stand and the point of standing upright on the recording paper.

The response is expressed as the 30:15 ratio. This is the longest R-R interval after standing (normally occurring around the 30th beat) divided by the shortest R-R interval after standing (normally around the 15th beat), as follows:

\[
\text{30 : 15 ratio} = \frac{\text{Longest R-R interval at about the 30th beat after standing}}{\text{Shortest R-R interval at about the 15th beat after standing}}
\]

Students may also be instructed to measure the time after standing at which these events occur, providing a further idea of normal physiological variation.

**Experiment 4: systolic BP response to standing.** The subject’s systolic BP should be measured at least twice while lying. It should be measured again after standing unsupported at 1 min and at 2 min. The arm should be outstretched so that the measurement takes place at the level of the heart (see below).

The result is expressed as follows:

Systolic blood pressure lying

\[
\text{systolic blood pressure standing after 1 min (in mmHg)}
\]

Diastolic BP may also be measured to allow the calculation of mean arterial pressure (MAP) and pulse pressure (see Wider Educational Applications).

For experiments 2 and 4, the inclusion of continuous beat-to-beat measurement of BP (for example, with the “Finapres” system) can be included when available, to more fully describe the characteristic cardiovascular responses to these procedures.

**Troubleshooting**

Experimental problems encountered may be concerned with general issues about accurate BP measurements, such as the cuff being...
too loose or an inability to hear clear Korotkoff sounds due to experience and/or background noise. Less familiar students should practice BP measurement before they begin the protocols. Subjects who may have not adequately rested immediately before HR measurement and with higher resting HRs may show less clear sinus arrhythmia, although it should still be present. This may be improved if students are allowed adequate rest time.

It is important that during recording of BP on standing that subjects reach out their arm to ensure that there is not a falsely elevated BP reading due to the hydrostatic pressure effect of the column of blood in the dependent arm. Inaccuracy may also be encountered in the 30:15 HR ratio if the rate is counted too early after standing or too rigidly on the 15th and 30th beats; it is intended that the maximum tachycardia and maximum decline in HR after the tachycardia are measured, which are not likely to be exactly on the 15th and 30th beats.

**Safety Considerations**

Subjects should be excluded for the following reasons:

1. Any cardiovascular or neurological disease
2. Caffeine or any other drug ingested before the practical
3. Smokers
4. Proliferative retinopathy

Students should also be warned against inflating the cuff too high or for too long.

**RESULTS**

**Expected Results and Evaluation of Student Work**

The calculated results from an individual may be presented in a table (see Table 1), which conveniently allows comparison between the subject’s results and those of borderline/abnormal subjects. It is also a valuable opportunity for the students to appreciate the variation of physiological measurements within a “normal” population by calculating a class average.

**Inquiry Applications**

**Question 1.** Based on the results you have obtained, comment on the cardiovascular autonomic function of your subject.

**ANSWER.** This allows students to go through the process of “critical” appraisal of the results, which usually concludes that the subjects are normal but also allows the students to see if any results are abnormal and to think about what might be the cause: genuine abnormality (extremely unlikely in the normal student population) or experimental error (much more likely). It is fairly common for students to get an abnormal reading from one of their measurements, and this can open a discussion as to the causes and the common practice of clinicians to use a battery of favored tests to build up a picture of the subject’s autonomic function rather than rely on a single test.

**Question 2.** How can you explain the mechanisms underlying the expected results and those you have obtained?

**ANSWER.** This requires a basic knowledge of autonomic cardiovascular reflexes applied to each test:

In **experiment 1**, throughout the respiratory cycle, there are varying degrees of inhibitory input acting on vagal motor neurones arising from a combination of inspiratory central neurones and reflex inputs from afferents such as lung stretch receptors and baroreceptors (3). The vagal inhibition is maximal during inspiration, resulting in the observed tachycardia, and of less influence during expiration, allowing greater vagal effects on HR, and therefore slowing during expiration. Therefore, this test primarily reflects parasympathetic control of HR via the vagus nerve.

It is common to observe a high degree of sinus arrhythmia in young people, and this tends to decline with age, although it is still usually present. It is also more marked in athletes who regularly engage in aerobic exercise and who have low resting HRs, reflecting high vagal tone (2).

In **experiment 2**, the use of this procedure as a teaching laboratory activity has been fully described recently (8). The response is commonly divided into four phases (8, 10, 12), relating mechanical and cardiovascular processes: in phase I, the strain and the additional intrathoracic pressure during the strain are transferred to the aorta, causing a transient increase in BP. As strain continues in phase II, venous return is reduced, and there is a resultant drop in cardiac output and BP. This results in a baroreceptor-mediated tachycardia and an increase sympathetic nerve and adrenal output, causing increased vascular resistance. There is then a rapid decline in BP as the strain is released in phase III, followed a few seconds later by phase IV, in which BP now rises in excess of basal levels, due to an increased cardiac output (via increased/restored venous return) in parallel with increased peripheral resistance due to persistence of the raised sympathetic-renal tone. Although this response belies several components of the reflex arc, including the afferent baroreceptor function and central components, it primarily reflects parasympathetic control of HR via the vagus nerve, as it is abolished by atropine but is unaffected by propranolol (4).

In **experiment 3**, increased hydrostatic pressure in the lower extremities immediately on standing allows ~500 ml of blood to pool in distensible vessels below the level of the heart, reducing venous return. The consequent reduction in BP activates a baroreflex-mediated tachycardia that peaks around the 15th beat but then declines until around the 30th beat in HR. Again, this reflects primarily the vagal control of HR (4, 12).

In **experiment 4**, the initial drop in systolic BP is normally counteracted as part of the baroreflex-mediated response to the blood volume displacement, so that pressure does not normally fall by >10 mmHg by the end of the first minute. This is mediated in part by increased sympathetically mediated peripheral vasoconstriction (5).

**Table 1. Results table**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
<th>Individual</th>
<th>Class Average (and Range)</th>
<th>Normal*</th>
<th>Borderline*</th>
<th>Abnormal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>deep breathing, beats/min</td>
<td>≥15</td>
<td>11–14</td>
<td>≤13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Valsalva ratio</td>
<td>≥1.21</td>
<td>1.11–1.21</td>
<td>≤1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 3</td>
<td>standing 30:15 ratio</td>
<td>≥1.04</td>
<td>1.01–1.03</td>
<td>≤1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 4</td>
<td>blood pressure (systolic) fall on standing, mmHg</td>
<td>≥16</td>
<td>11–15</td>
<td>≤10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data from Ref. 5.
Wider Educational Applications

As described above, these laboratory activities are primarily at the “methods” level of inquiry, where the questions to be answered by the investigations and the methods used are as prescribed by the teacher. The level of inquiry may be increased by posing the following question: “What further tests, invasive or noninvasive, may be suitable for the clinical assessment of autonomic cardiovascular control?” Students can then be directed to literature via scientific search sites, such as PubMed (http://www.ncbi.nlm.nih.gov/pubmed), which will expose them to other methods of autonomic assessment. On the noninvasive side, they may find other “pressor” or “depressor” interventions, many of which can also be used in the teaching laboratory, such as the cold pressor test (15), the handgrip test, mental stress test, and skin blood flow tests (1, 10–13). Venous occlusion plethysmography or laser Doppler for the assessment of skin and muscle blood flow or the cardiovascular response to tilt table tests (10, 12) require more specialized equipment, which may be available in some institutions. Students may then develop additions to the existing battery or a further battery of tests, with input from their teacher. Literature study of more invasive procedures, such as pharmacological interventions of BP, measurements of plasma catecholamines, and microneurography (18) can allow more detailed discussion of different aspects of cardiovascular reflex arcs.

As described in experiment 4, systolic BP is used to evaluate baroreceptor-mediated responses that are mediated mainly by sympathetic nerves to blood vessels. This is the standard clinical procedure (4) and is simple and quick. However, measurement of diastolic BP may also be incorporated, and MAP can also be calculated (diastolic BP + 1/3 pulse pressure). The teacher may then engage in more inclusive discussion of the control of MAP in the context of cardiac output and total peripheral resistance, along with a discussion of pulse pressure and the role of vascular reactivity (9, 14).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.D.J. and E.T. conception and design of research; C.D.J. drafted manuscript; C.D.J., S.R., and E.T. edited and revised manuscript; C.D.J., S.R., and E.T. approved final version of manuscript.

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