LABORATORY DEMONSTRATION OF BAROREFLEX CONTROL OF HEART RATE IN CONSCIOUS RATS

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We have developed a laboratory exercise that demonstrates arterial baroreflex control of heart rate (HR) in the conscious unrestrained rat, incorporating graduate level physiological topics as well as a hands-on exposure to conscious animal research. This demonstration utilizes rats chronically instrumented to measure cardiac output (CO), HR, and arterial blood pressure in response to agents that raise or lower blood pressure. The HR response to progressive increases or decreases in blood pressure is recorded, and a baroreflex curve is generated by plotting mean arterial blood pressure (MABP) vs. HR. Observation of altered CO allows for discussion of the relationship between MAP, CO, HR, stroke volume, and total peripheral resistance. Administration of arginine vasopressin demonstrates the ability of this hormone to alter the sensitivity of the baroreflex.

Throughout the demonstration, students answer questions from a handout about general cardiovascular physiology, specific pathways of agonists, and the baroreflex system, encouraging group and individual critical analysis of the results. Interpretation of the data reemphasizes lecture material and allows students to observe the baroreflex response in a physiological setting.

Key words: blood pressure; heart rate; cardiac output

In the cardiovascular system, the baroreflex is an important regulator of arterial blood pressure (5). The baroreceptor reflex mediates rapid changes in sympathetic and parasympathetic activity in response to changes in blood pressure (BP). The arterial baroreceptors sense changes in BP and send afferent signals to the nucleus of the tractus solitarius (NTS) in the medulla. There, the signals are integrated and effectively compared to a set point value for this variable. Projections from the NTS send out efferent signals through the sympathetic and parasympathetic nervous systems to effect an appropriate change in the controlled variable (Fig. 1). The baroreflex response is effective only for short-term changes in BP, as changes in pressure that are sustained for more than 15 minutes result in a resetting of the baroreflex set point and baroreceptor output (1, 2, 4, 13). This fast-acting, complex reflex system provides an excellent model to demonstrate the elegant cardiovascular regulation of BP and heart rate (HR) in a physiological setting.

For the past 10 years, we have conducted a baroreflex demonstration as part of a physiology course required for first-year M.S. and Ph.D. graduate students in biomedical sciences. Student surveys suggest that this demonstration greatly enhances their understanding of general cardiovascular function as well as of reflex control of BP. Exposure to conscious-animal experimentation and interpretation of the various signals
displayed on the chart recorder provide an opportunity for students to understand the methodology employed in the generation of physiological data. This experience allows the biomedical graduate student to develop an appreciation for the importance of physiological research of the past, present, and future that cannot be gained with a computer simulation. Studying live animals allows for interpretation of data obtained from an individual subject rather than from a group, as would be presented in a virtual laboratory demonstration. This distinction is important, because an individual may not yield the predicted results, a subtlety that would be lost in a computer-simulated presentation. Furthermore, observation of how conscious-animal experiments are actually performed can greatly increase a student’s appreciation of a study. The elegance of this preparation, coupled with the intricacies of working with a live animal, can have a profound effect on the student’s understanding of the topic.

Before participating in this laboratory demonstration, students have received lectures on this material and a handout describing the experimental protocols outlined below. The learning objectives for this exercise are to 1) understand how control systems theory relates to the maintenance of a relatively constant BP (Fig. 1); 2) know the functional components of the arterial baroreflex, i.e., sensors and their afferents, location, and function of the controller and the efferent limbs of the reflex (Fig. 6); and 3) be able to predict baroreflex-mediated responses to a sudden rise or fall in BP. Students observe the HR response to an acute fall in BP and an acute increase in BP as well as the HR response to two different vasoconstrictor agents. Students should come to the demonstration prepared to answer questions on this and related topics, including cellular signaling pathways, sympathetic and parasympathetic contribution to vascular tone, and anatomy of the baroreflex (for reviews see Refs. 6, 11, 12, 14).

METHODS

Surgical Preparation

All protocols and surgical procedures employed in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of New Mexico School of Medicine. The protocol for chronic instrumentation of rats has been described previously (7). Briefly, male Sprague-Dawley rats (250–350 g; Harlan Industries) are anesthetized with pentobarbital sodium (50 mg/kg ip), intubated, and artificially ventilated (Harvard Apparatus rodent ventilator). A thoracotomy is performed, and the thymus gland is retracted to expose the ascending aorta. A pulsed Doppler flow probe is secured around the ascending aorta for subsequent measurement of cardiac output (CO). The flow probes are constructed by forming Silastic cuffs around piezoelectric crystal assemblies (20 mHz, 1 mm diameter, Crystal Biotech). Probe leads are routed through the chest wall and then subcutaneously to the base of the neck, where they are exteriorized and housed in a protective plastic cap sutured to the skin. The chest wall is closed and evacuated, and animals are given topical and systemic antibiotics and allowed to recover. Five days after Doppler probe implantation, animals are anesthetized with a mixture of ketamine (91 mg/kg im) and acepromazine (0.9 mg/kg im). Polyethylene catheters (PE-10 and PE-50) are advanced into the abdominal vena cava and aorta via the femoral vein and artery, respectively. The catheters are routed subcutaneously and placed in the protective cap with the flow probe leads. Systemic and topical antibiotics are administered, and the animals are allowed ≈ 2 days to recover before the demonstration.
Experimental Setup
On the day of the demonstration, rats are placed in a Plexiglas container (23 × 14 × 10 cm) through which fresh air is continuously circulated. Fresh bedding covers the bottom of the chamber. The catheters and Doppler probe leads are fed out of the top of the box, and the catheters are opened and flushed with heparinized saline. The arterial catheter is connected to a Statham-Gould P23 Gb pressure transducer with the output amplified by a Gould Universal amplifier. Additionally, the pulsatile pressure wave form electronically triggers a tachometer that monitors HR. Doppler probe leads are connected to a Crystal Biotech Doppler flowmeter that electronically pulses the probe and processes the frequency shift into an output analogous to flow for measurement of CO. Pulsatile and mean arterial blood pressure (MABP), HR, and pulsatile and mean Doppler signals are continuously recorded on separate channels of a Gould RS 3800 chart recorder. All signals are simultaneously processed with an analog-to-digital converter and continuously displayed on a computer monitor by use of a data acquisition and analysis system (AT-CODAS, Dataq Instruments). The Doppler flowmeter further provides auditory output that facilitates the students’ assessment of changes in both CO and HR during various protocols. Animals are allowed 30–60 min to adjust to their environment, and experiments are initiated when rats demonstrate stable BP and HR.

Demonstration
Ideally, the student group size does not exceed six, which allows for close examination of the experimental setup by the students and minimizes the disturbance to the rat. Students are oriented to the equipment, and a brief overview of the surgical preparation of the animal and construction of Doppler probes is delivered. The pressure wave form is examined for observation of the dicrotic notch, and the Doppler wave form is examined for estimation of systolic and diastolic flow in the ascending aorta. The students are asked the following questions:

**Question 1:** What is the dicrotic notch?

**Answer:** The dicrotic notch is a feature of the arterial pressure wave form that occurs as the aortic valve closes at the onset of diastole. At this time, the ventricular pressure falls below aortic pressure and causes a dip in the pressure wave form.

**Question 2:** Would you expect a similar diastolic flow pattern in the ascending aorta and in a more distal vessel, e.g., a renal artery? Why?

**Answer:** In the ascending aorta, the diastolic flow approaches zero, as no blood is entering the aorta when the aortic valve is closed and the ventricle is filling. However, since the arterial system functions as a hydraulic filter, more distal vessels have a relatively constant flow, even during diastole. This occurs because the aorta and other large distensible arteries are able to store much of the systolic stroke volume (SV) as potential energy. This is known as the Windkessel effect, and during diastole these elastic arteries recoil, resulting in continuous flow in the more distal vessels (3).

**Protocol 1: HR response to acute changes in pressure.** To establish the baroreflex set point, baseline HR and MAP data are collected and plotted on baroreflex HR response graph (Fig. 2) before the experimental protocols are begun. Ideally, the resting HR and BP in the rat are ~350 beats/min and 100 mmHg, respectively. Students can discuss the difference in the rat HR with the human HR (~75 beats/min) and the inverse relationship between animal size and HR. With the animal at rest, we examine the HR response to acute changes in BP. The HR response to a progressive decrease in BP is determined by a short intravenous infusion of sodium nitroprusside (SNP; 10 μg/min), a drug that serves as an NO donor (Fig. 3). The animal is allowed to recover from this treatment until baseline BP and HR are achieved. To examine the effect of an elevation in pressure, an intravenous infusion of the α1-agonist methoxamine (MTX; 50 μg/min) is administered (Fig. 4). In this demonstration, the infusions of SNP and MTX last <5 min, thereby minimizing the risk of baroreceptor resetting. If there are concerns of resetting, bolus injections of SNP and MTX could be utilized in place of the infusion. This approach may provide for a more precise examination of the baroreflex response; however, it could add considerable time to the demonstration. HR is recorded at each 5-mmHg increment in BP above...
and below set point on the baroreflex HR response graph (see Fig. 2). During this protocol, students examine the changes in CO that can be observed on the chart recorder. Additionally, this is an ideal time to discuss the ability of the baroreflex to reset, as well as other possible factors that may be involved in longer-term changes in BP. Furthermore, students are asked to draw the relevant vascular smooth muscle-signaling pathways involved in the responses to SNP and MTX (Fig. 5) to review the cellular mechanism of action of these agents in the cardiovascular system.

Question 3: Why does BP change upon administration of these drugs?

Answer: Changes in total peripheral resistance (TPR) provide a measure of vasoconstrictor or vasodilatory responses to these drugs. Administration of SNP causes a decrease in TPR, therefore a decrease in MABP. As pressure decreases, there is a reflex increase in HR and a subsequent increase in CO. Administration of MTX causes an increase in TPR, therefore an increase in MABP and subsequent decrease in CO. The equations below demonstrate the relationship between MABP, CO, TPR, HR, and SV.

$$\text{MABP} = \text{CO} \times \text{TPR}$$

$$\text{CO} = \text{HR} \times \text{SV}$$
These relationships are discussed in the context of reflex control of BP.

**Question 4:** What are the sensors for the reflex change in HR observed in this experiment? Predict the neural output of these receptors associated with the responses to each drug.

**Answer:** These responses are sensed by the baroreceptors located in the aortic arch and carotid sinus. Baroreceptors are sensitive to stretch, and as pressure falls, as in the case of SNP administration, stretch and, hence, firing rate diminish. Treatment with MTX results in increased pressure, increased stretch and, therefore, increased firing of baroreceptors. The aortic and carotid baroreflex receptors send afferent signals through the aortic nerve and carotid sinus nerve, respectively. The aortic nerve projects via the vagus nerve, whereas the carotid sinus nerve travels via the glossopharyngeal nerve, both of which synapse within the NTS in the medulla.

**Question 4:** What are the predicted changes in sympathetic and parasympathetic output to the heart and vasculature associated with each agent?

**Answer:** In response to SNP, there would be a decrease in pressure and subsequent decreased firing of the baroreceptors (less stretch). The decrease in afferent signals results in diminished neural output from the NTS to the caudal ventrolateral medulla (CVLM) and from the CVLM to the rostral ventrolateral medulla (RVLM) (15). Neurons from the CVLM release the inhibitory neurotransmitter γ-aminobutyric acid; therefore, decreased CVLM signaling results in disinhibition of the RVLM and increased sympathetic firing through the intermediolateral (IML) gray column, causing increased sympathetic tone to the vasculature and increased HR. There is also a decrease in the parasympathetic output to the heart, via the vagus nerve, which causes a further increase in HR. Delivery of MTX elicits the opposite response, i.e., decreased sympathetic tone to the vasculature and decreased HR. There is little parasympathetic involvement in the systemic vasculature (Fig. 6).

**Protocol 2: response to arginine vasopressin.** While students are answering these questions from the handout and plot baroreflex curves, the animal recovers from the first protocol until stable baseline hemodynamic parameters have been reestablished. In the second protocol, we examine the response to a

![FIG. 6. Afferent and efferent pathways of the baroreflex. NTS, nucleus of the tractus solitarius; CVLM, caudal ventrolateral medulla; RVLM, rostral ventrolateral medulla; IML, intermediolateral gray column; NA, nucleus ambiguus.](image-url)
pressor dose of the peptide hormone arginine vasopressin (AVP). AVP elicits a pressor response when administered at a sufficient dose due to its actions on vascular smooth muscle. Interestingly, there also appear to be receptors for AVP within the area postrema (16), an area of the medulla that is devoid of an effective blood-brain barrier. Thus this region is exposed to circulating AVP. Furthermore, this region lies immediately adjacent to the NTS and sends to that site projections that can affect the function of the baroreflex (10). Circulating AVP has been shown to increase the gain or sensitivity of the baroreflex through its action in the area postrema (9). Here, we administer a bolus dose of AVP (50 ng iv) and record the hemodynamic response (see Fig. 2).

**Question 6**: Does the HR response to AVP lie on the pressor limb of the curve generated with MTX? If not, would you characterize the actions of AVP on the baroreflex as sensitization or desensitization?

**Answer**: AVP causes an increase in the gain, or sensitivity, of the baroreflex. The predicted response is a greater bradycardic response with AVP compared with MTX for the same change in MAP.

**Student Evaluations**

At the end of the demonstration, students are asked to fill out anonymously a questionnaire that was designed to assess the perceived value of this exercise (Table 1). We find that the demonstration is well received by the students and seems to enhance their overall understanding of this complex material. In the face of the consistently high approval ratings for this demonstration, it is noteworthy that this demonstration is not optional for the course: students are required to attend. The comment from one student reflects the general student sentiment in response to question 7:

“The demonstration reinforced lecture material and helped me remember the relationship between HR/BP and baroreflex because there was something tangible to remember.”

The major criticism of this exercise (question 8) is that it requires too much time (about 1.5 hours) to complete. There are also concerns with the group size if it gets too large, because students do not feel that they get the hands-on experience they expected.

**DISCUSSION**

Additional topics in reflex control of BP could be discussed as part of this demonstration. For example, the baroreflex is employed in cases of acute changes in BP or blood volume. If the cardiovascular response to hemorrhage is covered in lecture before the demonstration, many of the same principles examined here could be applied to a discussion on changes in blood volume as well. Investigation of the reflex response to a significant decrease in blood volume would reinforce the relationship between MABP,
Bainbridge and Bezold-Jarish re the atrial and ventricle volume receptors and the TPR, CO, HR, and SV. Furthermore, involvement of the Bainbridge and Bezold-Jarish reflexes could be discussed. Other related topics include long-term regulation of BP and the resetting of the baroreflex. Use of a longer-term infusion model of testing baroreceptor sensitivity would be ideal for demonstrating the ability of the baroreflex to reset.

This preparation could be further utilized to teach the fundamentals of autonomic pharmacology and regulation. For example, agents that would demonstrate adrenergic and nonadrenergic vasoconstriction/vasodilation and cardiac vs. vascular-acting agents could all be employed to expand the educational possibilities of this laboratory demonstration. However, due to time constraints, these alterations would likely necessitate scheduling additional demonstrations. Furthermore, this baroreflex demonstration can be coupled to an additional vascular demonstration utilizing isolated aortic ring segments to specifically demonstrate the importance of endothelium-dependent and independent vasodilators [see Gonzales et al. (8)].

Alternatively, this laboratory could be simplified for both time considerations and technical capabilities. Although inclusion of the CO response may help clarify some aspects of this demonstration, instrumentation for CO is technically difficult. An animal that has been poorly prepared or has incompletely recovered would detract from the significance of this exercise. A baroreflex HR response graph could be generated with an animal instrumented only with arterial and venous catheters. In this case, students would still have the benefit of hands-on experience in physiological experimentation and still grasp a better understanding of the relationship between BP and HR. Furthermore, the technical expertise and expense required for this preparation would be considerably less.

In summary, we have described a baroreflex laboratory demonstration that reinforces concepts in cardiovascular physiology typically taught in a first-year biomedical graduate curriculum. Students gain experience in the methods frequently utilized to generate physiological data. Although a computer-simulated laboratory demonstration may be appropriate for an undergraduate-level course, in this world of the increasing virtual laboratory it is crucial that biomedical graduate and medical students be exposed to conscious animal experimentation that fosters an appreciation of the important role of whole animal research in the study of physiology. Student surveys reveal that this laboratory exercise is well received by the students and is an effective learning experience.

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