Teaching principles of cardiovascular function in a medical student laboratory

Sanjaya Gupta, Thomas C. Westfall, Andrew J. Lechner, and Mark M. Knuepfer

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, Missouri

Submitted 23 September 2004; accepted in final form 18 January 2005

Gupta, Sanjaya, Thomas C. Westfall, Andrew J. Lechner, and Mark M. Knuepfer. Teaching principles of cardiovascular function in a medical student laboratory. Adv Physiol Educ 29: 118–127, 2005; doi:10.1152/advan.00052.2004.—We describe an animal laboratory using anesthetized swine to demonstrate the regulation of arterial blood pressure to second-year medical students at Saint Louis University School of Medicine (St. Louis, MO). The laboratory is designed to illustrate basic pharmacological and physiological concepts learned in the classroom. The specific learning objectives covered in this lab include maintenance of anesthesia, basic surgical technique including cannulation of blood vessels, understanding the measurement and significance of basic physiological parameters, premortem examination of in situ heart and lungs, direct cardiac massage and induction of ventricular fibrillation, understanding the fundamentals of the baroreceptor reflex, and cardiovascular responses to various pharmacological agents. Pharmacological agents used include epinephrine, norepinephrine, isoproterenol, atropine, prazosin, propranolol, acetylcholine, nitroprusside, and angiotensin II. The laboratory demonstration has proven effective in reinforcing the fundamental principles of cardiovascular physiology and autonomic pharmacology. By the completion of this experiment, students are expected to be able to: 1) describe the basics of maintenance of anesthesia in a live animal; 2) describe basic surgical technique; 3) observe the procedure for proper cannulation of blood vessels; 4) describe the proper method of controlling hemorrhage from a bleeding source; 5) describe the measurement and recording of four physiological parameters: mean arterial pressure from a pressure transducer, heart rate from an ECG, hindquarters resistance from Doppler measurement of femoral arterial blood flow, and cardiac contractility by calculating dP/dt from left ventricular pressure measured with a Millar transducer; 6) perform a premortem exam of the heart and lungs and appreciate the in situ cardiothoracic anatomy of the living animal; 7) assist in the induction of ventricular fibrillation and perform direct cardiac massage; 8) characterize the autonomic responses activated by the baroreceptor reflex; 9) describe the effects of the adrenergic agonists epinephrine, norepinephrine, and isoproterenol on cardiovascular parameters and construct a dose response curve for each agent; 10) describe the effects of the adrenergic antagonists propranolol and prazosin on cardiovascular parameters and explain how they affect cardiovascular responses to adrenergic agonists; 11) describe the difference between endothelium-dependent and endothelium-independent vasodilation using acetylcholine, nitroprusside, and atropine; 12) observe the pressor response of angiotensin II and describe why this response is not blocked by pretreatment with prazosin; and 13) participate in the collection and analysis of experimental data and the presentation of results.

cardiovascular laboratory; hemodynamics; cardiac contractility; arterial pressure regulation; adrenoceptors; skeletal muscle blood flow regulation; heart rate regulation

SECOND-YEAR MEDICAL STUDENTS at Saint Louis University School of Medicine (St. Louis, MO) have participated in an animal laboratory as part of the cardiovascular disease module for the past several years. This laboratory uses an anesthetized swine as the subject for experiments in regulation of arterial blood pressure conducted before 25–30 students. Under the guidance of veterinary doctors and registered veterinary technologists, students assist in dissecting blood vessels, inserting cannulas into arteries and veins, administering pharmacologic agents, and, at the end of the experiments, opening the thoracic cavity and inducing arrhythmias by manual compression of the heart.

Students have the opportunity to apply the physiological and pharmacological concepts they learned in the classroom and appreciate the complex, interrelated systems responsible for the control of blood pressure. Specific learning objectives covered in this lab include induction and maintenance of anesthesia, basic surgical technique, cannulation of blood vessels, control of hemorrhage, understanding the measurement and recording of physiological parameters, premortem examination of in situ heart and lungs, direct cardiac massage and induction of ventricular fibrillation, understanding of the autonomic innervation that underlies the baroreceptor reflex, and cardiovascular responses to various pharmacological agents. Agents studied include angiotensin II, endothelium-dependent and -independent vasodilators as well as both α- and β-adrenergic agonists and antagonists.

Although attendance is voluntary, this laboratory is always well received by >90% of the medical students who attend. This lab is an integral component of the second-year medical school curriculum, and for the vast majority of these students, this was their first opportunity to participate in an integrative physiology lab using an intact animal model. However, many medical schools have discontinued animal labs due to increasing costs of animals and equipment, shortage of qualified demonstrators, as well as pressure from animal rights activists. Approximately 50 medical schools continue to use such labs, while the majority of medical schools have now turned to alternatives, such as computer simulations and videotapes (6). For a list of medical schools that use animal labs in medical school curriculum, refer to the Physicians Committee for Responsible Medicine website: http://www.pcrm.org/issues/Ethics_in_Medical_Research/ethics_medlab_list. Although these alternatives are valuable supplements to classroom learning, they cannot serve as a substitute for an experience with a living organism (7).

MATERIALS AND METHODS

Protocols for this exercise were approved in advance by the Saint Louis University Institutional Animal Care and Use Committee.

Equipment

The following equipment was used for each animal in this experiment.

An anesthesia machine with anesthesia ventilator (Narkomed AV-E) equipped for medical oxygen and medical air (Reconditioned Narkomed Standard for Veterinary Use) was from North American Drager (Telford, PA).
A Mallinckrodt endotracheal tube (size 6.5 mm ID for most swine 20–25 kg), laryngoscope handle with no. 4 Miller blade, respirator tubing to make circuit system with respirator, standard veterinary mask for anesthesia induction, and isoflurane (general anesthetic) were used.

Polyethylene (PE)-200 tubing was 13 in. long, on a blunt 18-gauge needle for accessing the left femoral artery. The catheter introducer sheath with sideport and adjustable (preferable) hemostasis valve for Millar catheter placement into left carotid artery was 6.0- or 7.0-Fr. A 10-Fr silastic tubing 10-in. long on a 15-gauge blunt needle was used for the right jugular or left femoral vein line. Two three-way stopcocks were used.

Silk ligature 2-0 was used for securing cannulas to vessels and 0 silk or Vicryl suture was used for securing cannulas to skin.

Disposable pressure transducers (pressure monitoring kit) was from Baxter Healthcare (Deerfield, IL). The flow probe, size 5 mm was from Transons Systems (Cornell Research Park, Ithaca, NY). A catheter (size: 3-Fr diameter, 50-cm length) and transducer control unit was from Millar Instruments (Houston, TX).

The simultrace recorder and monitor (model VR-16) were from Honeywell (Morristown, NJ). A computer with data acquisition software (WINDAQ) was from DATAQ Instruments (Dayton, OH).

Drugs used were epinephrine, norepinephrine, atropine, isoproterenol, nitroprusside, propranolol, and prazosin.

Surgical equipment used was a scalpel no. 10 blade, needle drivers, right angle, hemostat, Metzenbaum scissors, DeBakey forceps, bone cutters, Weitlaner retractor, and Finocicott rib spreader.

Hespan plasma expander (500 ml per animal) was used.

Preparation of Animals

The pigs in this experiment were domestic swine and had an average weight of 20–25 kg. In the first year, seven pigs were used. One pig was studied before the first laboratory demonstration so that the protocol and duration could be reviewed and any potential difficulties could be addressed. The remaining six pigs were used for each group of ~25 medical students. Ideally, these groups could be smaller, but limitations on time and equipment prevented us from doing this. Keeping the groups relatively small allowed more students to participate in various aspects of the experiment. Six students volunteered to come 1 h before the beginning of the experiment to assist in the preparation of the animal. This allowed these students to gain some hands-on experience in basic surgical techniques during the preparation of the animal.

First, the pig was anesthetized via a mask induction using respiratory tubing connected to a standard veterinary mask placed over the pig’s snout. A mixture of isoflurane (4–5% initial concentration) and oxygen was given to anesthetize the pig. The animal was then placed in the supine position on the operating table, and its limbs were secured to the table. Once the animal was deeply anesthetized, intubation was performed using a size 6.5 mm ID cuffed endotracheal tube, and correct placement was confirmed by observing bilateral breath sounds using a stethoscope. The pig was ventilated using a Reconditioned Narkomed Standard for Veterinary Use using 2–3% isoflurane mixed with balanced medical grade oxygen. The respirator was set to deliver 10–15 ml/kg body wt of tidal volume and a peak inspiratory airway pressure of 16–18 cmH₂O. The end-tidal CO₂ was measured with the use of a capnometer and maintained between 3.7 and 4.0%, as corroborated by blood gases drawn intravenously. The CO₂ level was verified by analyzing periodic arterial blood gas samples. The anesthesia level was titrated “to effect,” and the level of anesthesia was monitored by testing the corneal reflex, jaw tone, and limb withdrawal. If any of these reflexes were elicited, the concentration of anesthetic was increased until the response subsided. Typically, the level of expired isoflurane varied from 2.8 to 3.0%. The exercise allowed students to observe how much anesthesia was necessary to keep the pig in a relaxed and pain-free status without causing respiratory arrest. This emphasized the humane treatment of experimental animals as well.

If desired, another anesthetic regimen may be used. Etomidate or the combination of ketamine and xylazine as a preanesthetic treatment before isoflurane administration at a lower dose in combination with pentobarbital can be used. These anesthetic regimens may reduce the suppression of baroreflex function associated with isoflurane alone. Any improvement in baroreflex function is likely to make the laboratory more interesting as a teaching experience.

ECG leads were attached to the four limbs of the pig and the ECG was continuously monitored on a computerized data acquisition program as well as a paper chart recorder. Students observed the recorded ECG as they learned to recognize basic arrhythmias and were reminded of their significance.

Subsequently, cannulas were inserted in femoral blood vessels for monitoring of blood pressure and the injection of pharmacological agents. This was an important opportunity for students to recognize the difference between arterial and venous pressures and, consequently, the risk for spontaneous bleeding. When hemorrhage occurred, students were shown how to control bleeding by applying direct pressure. Students also experienced firsthand the difficulty in manipulating vessels as they inserted the cannulas. When they secured each cannula with surgical ligatures, students discovered that a reliable knot halts bleeding, whereas an unreliable knot allowed continued seepage of blood. This experience serves as an introduction to the task of inserting an intravenous cannula in a human.

The cannulas were inserted using a cut-down technique. By starting in the right inguinal area, the students located the femoral pulse, and a 3- to 5-cm midventral skin incision was then made using a scalpel. With the use of a curved hemostat, blunt dissection was performed through the subcutaneous tissues until the femoral artery and vein were located. With the use of a right angle, blunt dissection was performed medial and lateral to each vessel. The vessels were also freed from underlying fascia by hooking the right angle behind the vessel and spreading the ends of the right angle. Care was taken at this step because the vasa vasorum in pig vessels is prominent and the vessel and prone to rupture and bleed with minimal trauma. Consequently, the risk for spontaneous bleeding.

Once each vessel was exposed and separated, two 2-0 silk ties were looped around the femoral artery. One silk tie was tied at the distal end of the vessel while the other one was looped but not tied at the proximal end of the vessel. Applying upward pressure on the proximal tie with one hand allows occlusion of blood flow while a small incision is made with Metzenbaum scissors or a smaller variety of surgical scissors. It was imperative to make the incision small such that the tunica media and intima of the vessel were transected on one side and preserved on the other side. This created a small hole in the vessels (~1–2 mm in size) that was used to insert the end of the saline-filled cannula. By maintaining upward pressure on the proximal tie with one hand, the cannula was inserted into the small opening in the femoral artery. Slowly, the cannula was advanced proximally until the end of the cannula reached the proximal tie. The proximal tie was then relaxed, and the cannula was advanced past the tie. Once the cannula had been advanced ~3–4 cm past the proximal tie it was in the correct position. The proximal tie was then tied to secure the cannula in place inside the vessel. The distal end of the cannula was secured by tying suture around an exposed distal end of the cannula and looping it around the cannula several times before suturing it to the pig’s skin. Thus both proximal and distal ends of the cannula were secured. The other end of the cannula was connected to a pressure transducer after calibrating the amplifier.

The femoral vein was cannulated using a similar approach. Once the femoral vein cannula was secured, the line was flushed with additional heparinized saline (10 U/ml heparin) and connected to a 1.0-liter bag of sterile 0.9% NaCl (saline). Fluids were administered at a rate of 5 ml/kg−1·h−1. In addition to intravenous fluids, pharmacologic agents were administered via this cannula. Once both vessels
were cannulated, the skin was closed (leaving space for the cannula to exit) by using several simple interrupted sutures.

On the contralateral side, the femoral vessels were exposed using the cut-down technique described above. Once the femoral artery was exposed, a 5-mm transit time flow probe (Transonics, Ithaca, NY) was placed over this vessel. This was used to measure femoral arterial blood flow as an estimate of skeletal muscle blood flow.

The left carotid artery was also cannulated using the cut-down technique. The carotid pulse was palpated just lateral to the trachea. A skin incision was made, and the vessels were exposed as described above. The carotid sheath was opened and the carotid artery was separated from the jugular vein. An introducer was inserted and used to assist the advancement of the Millar transducer into the left carotid artery and then into the left ventricle to measure ventricular pressure. Due to the anatomy of the pig vasculature, the left carotid artery was used, because swine have a common carotid artery that bifurcates into the left and right carotid arteries. Experience with both approaches showed that it was much easier to introduce the Millar transducer into the left ventricle from the left carotid artery as opposed to the right carotid artery.

At this point, the animal was instrumented fully for the ensuing experiments. To stabilize, the animal was allowed to recover for ~20 min, during which time 500 ml of Hespan plasma expander were infused intravenously to support arterial pressure, normally low in anesthetized swine. Concurrently, the remaining students arrived, and the anesthesia induction and surgical preparation were described to those students who did not participate in the surgery.

**Experimental Procedure**

At this time, the five baseline parameters (Table 1) were noted from continuous recordings using the various equipment and posted on a large dry erase board visible to all attending students.

**Baroreceptor reflex.** Initially, attempts were made to demonstrate the baroreceptor reflex by artificially occluding the carotid arteries. When occluded, the carotid sinus baroreceptors normally sense a drop in arterial pressure, resulting in reflex activation of the sympathoadrenal system to increase blood pressure. This reflex increase is due to generalized vasoconstriction and to a lesser extent by increased cardiac output. In addition, there is also a positive chronotropic and inotropic effect as well as an increase in atrioventricular conduction and total peripheral resistance. However, it was difficult to properly demonstrate the baroreceptor reflex due to the relatively deep level of anesthesia. In subsequent years, the carotid arterial occlusion was omitted from the experiment, although the response to carotid occlusion and its activation of the baroreceptor reflex is still described to the students to remind them of its actions and the effects of anesthetics.

**Pharmacological manipulations.** Initially, bolus infusions of a series of agonists were performed (Appendix A, top see Table 2 for dosages). Before each dose of drug was infused intravenously, the animal’s arterial pressure, ventricular pressure, heart rate, and iliac blood flow were measured and written on a large dry erase board so all students could observe and record the values. Students discussed expected responses of each parameter to the agonist and predicted one variable they considered the most likely to change in response to the particular agonist. When the peak change in that particular variable occurred (usually 30–60 s later), the student monitoring the variable would alert the other students recording parameters to ensure that all the data were obtained simultaneously. Values were recorded by students, and the predictions for each parameter were discussed. Meanwhile, the intravenous infusion cannula was carefully flushed with saline after each drug to avoid inadvertent mixing of drugs with subsequent dosing.

After recording responses to agonists alone, atropine was administered after students predicted the effects. This antagonist was used first because, in a baroreflex-depressed state due to anesthesia, little change in resting variables is observed. Subsequently, we administered catecholamines, acetylcholine, and nitroprusside (Appendix A).

Either pranoproanol or prazosin was then given (Appendix A). Students again predicted responses to agonists allowing them to understand the extent of α- and β-adrenoceptor activity of norepinephrine and epinephrine. In addition, because responses to selective agonists were typically attenuated but not blocked, the concept of competitive receptor antagonism previously discussed in lecture was reviewed. Finally, after all three antagonists were administered, students were asked to predict the effects of several agonists and to predict which agonists should still elicit cardiovascular responses.

During some experiments, the combination of pharmacologic agents produced occasional arrhythmias. When this occurred or, in some cases, was anticipated, lidocaine (1–3 mg/kg body wt) was given during baseline monitoring to ensure a normal sinus rhythm.

**Direct Observation of the Heart**

Once the pharmacologic experiments were complete (usually 90–120 min later), the students and instructors opened the thoracic cavity using a midsternal thoracotomy. An incision was made along the left border of the sternum using a scalpel. Once the sternum was exposed, bone cutters were used to open the thoracic cage. It was important to not cut too laterally to avoid injury to the internal mammary arteries that lie in close proximity to the sternum. Rib spreaders were used to further open the thoracic cavity and provide better visibility. The heart was exposed by grasping a piece of the pericardium and making an incision with Metzenbaum scissors.

### Table 1. Baseline parameters and instrumentation

<table>
<thead>
<tr>
<th>Baseline Parameter</th>
<th>Means for Recording Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pressure</td>
<td>Femoral artery catheter</td>
</tr>
<tr>
<td>Left ventricular pressure</td>
<td>Intraventricular Millar transducer</td>
</tr>
<tr>
<td>Electrocardiogram</td>
<td>Four skin electrodes</td>
</tr>
<tr>
<td>Respiration and O₂ saturation</td>
<td>Respirator settings and pulse oximetry</td>
</tr>
<tr>
<td>Skeletal muscle blood flow</td>
<td>Femoral artery flow probe</td>
</tr>
</tbody>
</table>

### Table 2. Agonists and dosage of drug infused intravenously

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist alone</td>
<td>0.1, 0.3, 1.0, 3.0</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1.0, 3.0</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>3.0</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>0.3</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>1.0 (3.0)</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>5.0</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>5.0</td>
</tr>
<tr>
<td>Agonists after atropine</td>
<td></td>
</tr>
<tr>
<td>Atropine alone</td>
<td>1.0 or 2.0</td>
</tr>
<tr>
<td>+ Epinephrine</td>
<td>1.0</td>
</tr>
<tr>
<td>+ Norepinephrine</td>
<td>1.0</td>
</tr>
<tr>
<td>+ Acetylcholine</td>
<td>5.0</td>
</tr>
<tr>
<td>+ Nitroprusside</td>
<td>5.0</td>
</tr>
<tr>
<td>Agonists after atropine and prazosin</td>
<td></td>
</tr>
<tr>
<td>Prazosin alone</td>
<td>100</td>
</tr>
<tr>
<td>+ Epinephrine</td>
<td>1.0</td>
</tr>
<tr>
<td>+ Norepinephrine</td>
<td>1.0</td>
</tr>
<tr>
<td>+ Angiotensin II</td>
<td>0.3</td>
</tr>
<tr>
<td>Agonists after atropine, propranolol, and prazosin</td>
<td></td>
</tr>
<tr>
<td>Propranolol alone</td>
<td>1.0 (2.0)</td>
</tr>
<tr>
<td>+ Epinephrine</td>
<td>1.0</td>
</tr>
<tr>
<td>+ Norepinephrine</td>
<td>1.0</td>
</tr>
<tr>
<td>+ Angiotensin II</td>
<td>0.3</td>
</tr>
<tr>
<td>+ Isoproterenol</td>
<td>1.0 (3.0)</td>
</tr>
</tbody>
</table>

Dosages are in micrograms per kilogram body weight of each animal.
After dissecting gloves and scrubs were made readily available, students were asked to identify the anatomy of organs in the thoracic cavity and the large vessels in the cardiopulmonary circulation. Students were also encouraged to palpate the heart to estimate the strength of the contraction of the heart and to observe the lungs expanding with each breath. Invariably, this experience fascinated the students. For many, this was their first opportunity to manipulate a beating heart or to palpate expanding lungs with their own hands. This experience allowed students to realize the amount of effort needed during cardiopulmonary resuscitation to mimic normal respirations and cardiac contractions.

RESULTS

Results from 16 pigs studied in the pig laboratory over the past 4 yr have been presented here, as well as examples of individual responses. Results are shown for the pharmacologic manipulations described above (see APPENDIX A). In the figures, the combined results are depicted as the change (± SE) in a specific response elicited by the agonist before and after treatment with antagonists. The parameters depicted include mean arterial pressure, left ventricular contractility (dP/dt), heart rate, and hindquarters vascular resistance. Students were taught that the arterial pressure and hindquarters blood flow could be used to calculate changes in vascular resistance by Ohm’s law (arterial pressure/hindquarters blood flow = hindquarters vascular resistance). Changes in the hindquarters vascular resistance were used to approximate vascular responses in skeletal muscle.

Agonists Alone: Epinephrine

Epinephrine is a potent direct agonist of α1-, α2-, β1-, and β2-adrenergic receptors (4). At low concentrations (0.5 µg/kg), the β2-effects predominated over the α-effects, and vasodilation of skeletal muscle beds occurred (Fig. 1). At intermediate epinephrine concentrations (0.1 and 0.3 µg/kg), the β2-effects still predominated, as evidenced by the consistent vasodilation in the hindquarters vasculature. However, the α1-receptors were also stimulated, resulting in vasoconstriction in skin and viscera. In addition, there was a β1-mediated increase in heart rate and cardiac contractility leading to an increase in arterial pressure. At high concentrations of epinephrine, the α1-effects are evident as a large increase in arterial pressure accompanied by positive inotropic and chronotropic effects on the heart via β1-receptors. The increasing recruitment of α1-receptors over β2-receptors was also evident as a reduced vasodilation in the hindquarters vasculature.

Agonists Alone: Norepinephrine

Norepinephrine is a direct agonist of α1-, α2-, and β1-receptors. Compared with epinephrine, norepinephrine showed equal potency at the α- and β1-adrenergic receptors, although epinephrine was 10–50 times more potent than norepinephrine at the β2-receptor (5). According to current understanding of the pharmacology of norepinephrine, this produced vasoconstriction of skin and viscera vasculature (α1-receptor effect) without a significant vasodilation of the skeletal muscle vasculature (β2-receptor effect). This was evident as a significant increase in arterial pressure with minor changes in hindquarters resistance (Fig. 2). There was also an increased dP/df (positive inotropic effect) similar in magnitude to that caused by epinephrine (0.3 and 1 µg/kg). However, the tachycardic (positive chronotropic) effect was not as great for norepinephrine compared with epinephrine. This may be due to a larger increase in arterial pressure seen with norepinephrine, which produces a greater reflex vasoconstriction. This, in turn, may have attenuated the tachycardia.

Effects of Selective Antagonists on Responses to Epinephrine

Figure 3 compares the effects of 1 µg/kg of epinephrine preceded by selective antagonists, including atropine (M), propranolol (B) and prazosin (A). Because atropine competes
with acetylcholine for binding sites on muscarinic receptors in the heart and smooth muscle the primary effect of atropine was tachycardia, although there was a transient slowing of the heart rate by 4–8 beats/min with average clinical doses (0.4–0.6 mg). Usually there were no accompanying changes in blood pressure or cardiac output (1). Atropine blocked vagal tone but revealed that despite the vagolytic properties of isoflurane a greater increase in heart rate was observed due to the lack of baroreflex-mediated vagal activation.

Propranolol is a nonselective β-adrenergic antagonist that has no intrinsic sympathomimetic activity. Thus propranolol’s effects depend on the degree of stimulation of β-adrenoceptors.

Fig. 2. Norepinephrine (NE; 0.1, 0.3, and 1 μg/kg body wt iv) evoked a dose dependent increase in MAP more potent compared with Epi. The enhanced pressor response was due to a smaller decrease in HqR, despite a decrease in the tachycardic (increased HR) effect. NE had little effect on HqR due to its weak agonist properties on α1- and β2-adrenoceptors.

Fig. 3. The increase in MAP elicited by 1 μg/kg body wt Epi was enhanced by muscarinic blockade (M) with atropine (1 mg/kg body wt iv) possibly due to greater increases in contractility (dP/dt); β-adrenoceptor blockade (B) with propranolol (2 mg/kg body wt iv) reduced the increase in contractility (dP/dt) and prevented hindquarters vasodilation, resulting in a smaller pressor response. Pressor response was prevented by α-adrenoceptor blockade (A) with prazosin (100 μg/kg body wt iv). Prazosin alone also revealed the substantial β-mediated skeletal muscle vasodilation elicited by Epi. Increase in contractility and HR was reduced selectively by β-adrenergic blockade. Responses remaining after muscarinic, and α-, and β-adrenoceptor blockade (M,B,A) indicated that the antagonists did not completely prevent responses presumably due to incomplete receptor blockade.
by endogenous catecholamines. When endogenous sympathetic tone was intact, propranolol decreased heart rate and cardiac output by blocking β₁-adrenoceptors. Propranolol also blocked vasodilation induced by β₂-receptors and caused an increase in peripheral resistance (4). When propranolol was added after atropine, the positive inotropic and chronotropic effects (β₂-effects) of epinephrine were blocked (Fig. 3) as evidenced by significant decreases in heart rate and dP/dt. Similarly, any vasodilation (β₂-effects) produced by epinephrine was also blocked, as illustrated by the increase in hindquarters resistance. The pressor response (due to β₁-adrenoceptors) seen with epinephrine was not antagonized (Fig. 3).

By inhibiting β₁-adrenoceptors, prazosin reduced blood pressure by reducing peripheral vascular resistance. The magnitude of this effect was dependent on the concentration of endogenous catecholamines. Administration of prazosin alone usually had no effect on the heart rate (4). Concepts of passive vasodilation and anesthetic-induced increases in plasma catecholamines were discussed with the students during the demonstration. Addition of prazosin to atropine blocked the vasoconstrictive effect of epinephrine, producing a decrease in mean arterial pressure and hindquarters resistance (Fig. 3) and illustrating the classic phenomenon of “epinephrine reversal.” Without propranolol, the β₁-mediated increase in heart rate and contractility caused by epinephrine were preserved. However, when propranolol preceded epinephrine, atropine, and prazosin, these β₁-effects were inhibited and decreases in heart rate and dP/dt were observed.

Effects of Selective Antagonists on Responses to Norepinephrine

Figure 4 illustrates the combined effects of norepinephrine administered after the same set of selective antagonists used with epinephrine. Owing to the pressor effect of norepinephrine via α₁- and β₁-effects, arterial pressure decreased following the administration of propranolol as well as after prazosin. Propranolol also produced a decrease in contractility and heart rate. Norepinephrine alone had little effect on hindquarters resistance. However, the addition of prazosin revealed a pronounced α₁-mediated vasoconstrictor response. In contrast, the addition of prazosin revealed a small component of norepinephrine-induced β₂-mediated vasodilation.

Isoproterenol is a potent nonselective β-agonist that has very low affinity at α-adrenergic receptors. Therefore, isoproterenol induced a β₁-mediated increase in heart rate and contractility as well as a β₂-mediated vasodilation in the absence of any vasoconstrictive effects (4). As shown in Fig. 5, isoproterenol decreased mean arterial blood pressure and peripheral vascular resistance and also exerted direct positive inotropic and chronotropic effects. Propranolol pretreatment attenuated β₁-mediated effects of isoproterenol on the heart, producing decreases in heart rate, contractility, and arterial pressure as well as a β₂-mediated hindquarters vasodilation. As expected, prazosin pretreatment had little effect on these responses to isoproterenol.

Effects of Selective Antagonists on Responses to Acetylcholine or Nitroprusside

The administration of a bolus intravenous dose of acetylcholine normally mimics the increased vagal tone seen during a baroreceptor reflex; and is short-lived because of rapid

Fig. 4. Increase in MAP in response to NE is dependent on both α- and β-adrenergic receptors. β-blockade (B) with propranolol reduced the increase in dP/dt and blocked the increase in HR. NE appeared to have little effect on the HqR but β-adrenergic blockade revealed a potent vasoconstrictor response. In the opposite manner, α-blockade revealed a small NE-induced hindquarters vasodilation, although this was substantially less than with Epi.
hydrolysis of acetylcholine by plasma cholinesterases. As seen in Fig. 6, acetylcholine decreased heart rate, contractility, and hindquarters vascular resistance, effects that were prevented or reversed by atropine pretreatment. Nitroprusside acts as a vasodilator by releasing nitric oxide. It also produced a depressor response that led to a decrease in arterial pressure, hindquarters resistance, contractility, and heart rate. In contrast to acetylcholine, depressor responses to nitroprusside were not altered by atropine pretreatment. The concepts of endothelium-dependent and endothelium-independent vasodilation were reviewed with students during this experiment.

The complexities underlying the negative inotropic effects of acetylcholine were more difficult for students to understand. Acetylcholine elicits negative chronotropic and inotropic effects due to the opening of potassium channels. In a conscious animal, the decrease in contractility would not be expected but occurs in

---

**Fig. 5.** The selective β-agonist, isoproterenol (Isop), elicited a depressor response (decrease in MAP) due to β-adrenoceptor-mediated vasodilation in the skeletal muscle. Iso also elicited an increase in HR and contractility mediated by β-adrenoceptors.

**Fig. 6.** Administration of acetylcholine (ACh) elicited a depressor response associated with decreases in HR, dP/dt, and HqR. These effects were prevented or reversed by muscarinic receptor blockade (M) with atropine (1 mg/kg body wt iv). Nitroprusside (NP) also produced a depressor response due to vasodilation and reduced contractility. In contrast to ACh, depressor responses to NP were not altered by atropine pretreatment.
the anesthetized animal because sympathetic tone is elevated, particularly circulating catecholamines from the adrenal gland.

**DISCUSSION**

Blood pressure is determined by three primary factors: heart rate, stroke volume, and arteriolar caliber. In this experiment, students have the opportunity to examine in real time the interplay of these three factors and observe how endogenous and exogenous pharmacological agents modulate these factors to produce changes in blood pressure. This experience in an integrative physiology lab is a valuable supplement to classroom learning that is difficult to adequately reproduce by alternative methods such as computer simulations or taped demonstrations. It also became clear early in the development of this exercise that the laboratory offers a considerable amount of unstructured time for discussing basic principles because different students were asked to predict responses. This solidified many ideas previously memorized by students from lectures alone.

The measure of success for any teaching tool should include both students' perception of the exercise and the relative improvement in exam performance in this field. From the perspective of the students, this exercise has rated an average of 3.7 on a scale from 1 (strongly disagree or unacceptable) to 5 (strongly agree or excellent), including 1 yr when it received the highest score of any component of the course. In all years, it was above the average score for the various components of the course. On average, 20.5% of students rated the experience as excellent (5 of 5). In the present year (2004), the exercise was optional and limited to only two pigs. Of the students attending, 43% rated it excellent (5 of 5).

Assessment of the laboratory's contribution to student assimilation and content mastery is more difficult because we have instituted several changes to improve the understanding of autonomic function. These include adding a 1-h refresher course on autonomic agents covered in the previous year, a 1-h review of the swine laboratory results presented to the entire class, and two patient simulator laboratories (one in the first year and one during this course) to teach and reinforce these fundamental principles. Subjectively, at least, these students are considerably better prepared for examinations.

This exercise is not without limitations. There are substantial costs to house and maintain animals and to purchase and maintain the necessary equipment. In addition, this exercise requires more faculty time to organize and oversee these experiments. However, this exercise is a unique opportunity to effectively teach several important concepts, and the students always rate this experience highly. Another limitation is that a medical school must have faculty with experience in performing nonsurvival surgery and conducting live animal experiments. However, most academic medical centers have clinical faculty that would be willing to assist in this exercise.

This was the first opportunity for many students to perform surgery. The skillful use of both a sharp scalpel as well as blunt dissection using hemostats allowed students to gain practical experience in basic surgical technique. Although this limited experience is not sufficient to master surgical skills, it is an important first experience that often motivated students to explore an interest in surgery.

Faculty members conducting the exercise spent considerable time emphasizing the relationships between pressure, flow, and resistance. This is a fundamental concept in our understanding of cardiovascular function that is often confusing to students (2). With each drug administered, the changes in measured flow and pressure are used to calculate the change in vascular resistance such that the primary concepts are well understood before the end of the experimental protocol.

When the thoracic cavity was opened, students directly observed the correlation between cardiac contractions and the ECG tracing on the monitor. By manually compressing the heart, arrhythmias were produced and students noted how they appeared on the monitor as well as which drug was needed to return the heart to a normal sinus rhythm. Before the animal was euthanized with a bolus of potassium chloride, ventricular fibrillation was manually induced so students could correlate the physiological parameters with the loss of contractile strength seen in the fibrillating myocardium.

This experience also allowed students to manipulate tissues in situ and view anatomy in a live animal. When students dissect a cadaver in anatomy, they do not have the experience of following pulsation to find a blood vessel or facing the threat of sudden hemorrhage if they accidentally transect a vessel. In addition, the color and consistency of different tissues and organs is more apparent in the living flesh than in the cadaver.

The laboratory also allowed students to observe and evaluate the effects of anesthesia. Students noted that normal baroreflexes were suppressed in an anesthetized animal. Because the level of anesthesia changed over time, they had the opportunity to participate in monitoring the depth of anesthesia using multiple indexes. Students later observed these same phenomena in the operating room during their clinical clerkship, and this laboratory served as important introduction to those concepts.

During clinical clerkships, medical students do not have the opportunity to directly administer drugs to patients. Even as residents, it is more common for drugs to be given by nurses once an attending physician has written the order. Thus this lab provided a unique opportunity for students to administer graded doses of powerful pharmacological agents and to observe their immediate direct and indirect effects and the duration of the responses.

In addition, these experiments also involved the administration of specific antagonists to illustrate the mechanism of action of the agonist drugs. During their medical education, students often attempt to learn the mechanism of action of various agents by rote memorization without an appreciation for how this knowledge was acquired. By observing how specific pharmacologic antagonists alter the physiological effects of an agonist, the students gained firsthand experience of how a variety of pathways and receptors are responsible for regulation of blood pressure. This experience provided a more meaningful and lasting understanding of each pharmacologic agent, as well as an appreciation of how these drugs might be used to control blood pressure in a patient. While computer simulations of these experiences are helpful (3), the use of a live animal proved particularly stimulating to the students.

This lab also provided students with the opportunity to collect and analyze experimental data and to present results. These students eventually will read and interpret the medical literature to stay abreast of the latest medical knowledge. By participating in the collection and interpretation of such data, the students will better appreciate the experimental basis of contemporary medical pharmacology. Finally, by critically examining their own results, students learn to evaluate the results of others and to assess the putative significance of published findings.
APPENDIX A: SEQUENCE OF EXPERIMENTS

Agonists alone:
- Epinephrine
- Norepinephrine
- Phenylephrine
- Angiotensin II
- Isoproterenol
- Acetylcholine
- Nitroprusside

Agonists after atropine:
- Atropine
  - Epinephrine
  - Norepinephrine
  - Acetylcholine
  - Nitroprusside

Agonists after atropine and prazosin:
- Atropine
  - Prazosin
    - Epinephrine
    - Norepinephrine
    - Angiotensin II

Agonists after atropine and propranolol:
- Atropine
  - Propranolol
    - Epinephrine
    - Norepinephrine

Agonists after atropine, propranolol and prazosin:
- Atropine
  - Propranolol
    - Prazosin
      - Epinephrine
      - Norepinephrine
      - Angiotensin II
      - Isoproterenol

ACKNOWLEDGMENTS

The authors thank Wanda Morgenthaler, Jennifer Meyer, and Drs. George Vogler and Gregory Smith for assistance preparing and conducting the laboratory exercise. We also thank the hundreds of medical students at St. Louis University who participated and whose comments helped to improve this laboratory exercise.

Present address of S. Gupta: Univ. Hospitals of Cleveland, Dept. of Internal Medicine, 11100 Euclid Ave., Cleveland, OH 44106.

GRANTS

This work was supported by the Saint Louis University School of Medicine and United States Public Health Service Grant DA-05180.

REFERENCES


