Chapter 2

Vascular Cannulation of the Rat

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After receiving a Master's degree in biology from Emory University in 1969, I taught general biology and zoology at Mercer University in Macon, Georgia. In 1971 I returned to Emory to continue graduate studies in the Department of Physiology. It was there, working under Dr. J. L. Kostyo, that I developed an interest in endocrinology. I received the Ph.D. in physiology from Emory in 1975, and joined Drs. C. J. Goodner and Donna Koerker for postdoctoral training at the University of Washington in Seattle. I remained at the University of Washington as a Research Associate and Research Assistant Professor until 1981, when I accepted a position in the Department of Biology at Virginia Commonwealth University, in Richmond. I am now an Assistant Professor in this department. My research interests are in the areas of neuroendocrinology and metabolism. I am investigating the role of metabolic substrates in the regulation of neural function and peptide hormone secretion.

I express my appreciation to Dr. Donald Clifton of the University of Washington in Seattle for his advice in the development of this technique.

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Introduction

The techniques described here were adapted from a modification by Dr. Donald Clifton of the methods of Terkel and Urbach (1974). The objectives of this laboratory exercise are (1) to demonstrate a technique for intravenous infusion and blood sampling of conscious, freely moving rats, and (2) to introduce the student to general surgical procedures, e.g. anesthesia, dissection of a living specimen, internal suturing and wound closure. Unlike other common methods of blood collection that require either anesthesia or restraint, this method permits blood withdrawal from unstressed animals. The method is particularly useful for correlation of blood levels of hormones and metabolic substrates with behavior. Since the method permits multiple daily sampling in one animal, it has been used successfully in the study of endocrine rhythms. One example of the importance of chronic cannulation procedures is a study by Tannebaum and Martin (1976) demonstrating rhythmic fluctuations, at approximately 3 hr intervals, of growth hormone (GH) levels in rats. They observed peaks of GH that were often greater than ten times the basal concentrations, thus explaining the variability among previous acute studies in which a constant level of basal secretion was assumed.

This exercise is designed for advanced undergraduate students in biology, physiology or psychology, and graduate students interested in research on living animals. Approximately 2.5 hrs are required to perform the exercise. After the materials have been assembled, the preparation time for the exercise is minimal. Approximately 5 min per surgery is required to make one cannula and modify the needles that are used for inserting the cannula and withdrawing the blood sample.

Instructors' Materials

Materials

Rats (preferably 220–250g) housed individually
Physiological saline (0.85% NaCl)
Heparinized saline (250 units heparin/100 cc saline)
Animal hair clippers
Absorbent paper or other protective paper for covering surgery table
Rulers (10 to 15 cm)
1 cc syringes
Masking tape
Modified 23ga needles (see below)
Nylon fishing line, 30# wt.
Desk lamps or Tensor lamps or any light for the surgery table

Quick-drying epoxy

Needle connectors (23ga needles with sharp tip filed smooth)

Wound clips

One scalpel

2 forceps—straight or curved, with tapered tips (not a locking tip)

One hemostat or needle holder or wound clip applicator

5–0 silk suture with attached curved needle (coarser silk suture may be used if necessary)

Cannulae—20cm of either polyethylene—50 (P.E. 50) or Silastic tubing (0.02 inch I.D., 0.037 inch O.D., Dow-Corning #602–037) glued to a rectangular piece of silastic sheeting (Dow-Corning 500-3, non-reinforced). Glue = Dow-Corning Silastic Medical Adhesive Silicone, S91. (This glue requires 24 hours to cure. Other glue may be used if the cannulated animals are not going to be used in experiments that would be affected by tissue reactions to the glue.)

All surgical instruments, needles, heparin, and polyethylene tubing can be obtained from American Scientific Products, 1088 Metro Court, Maryland Heights, MD 63043. Epoxy, tape, and fishing line are found at local hardware and department stores. Silk suture, silastic tubing, medical silicone, and silastic sheeting may be ordered from Dow Corning through local hospital supply companies, such as Whitaker-General Medical, 3001 E. Parham Rd., Richmond, VA 23261.

Preliminary Procedures

To make the cannulae: Cut a piece of silastic tubing 20 cm in length. Cut a piece of silastic sheeting 1 × 0.5 cm and fold in half. The sheeting is used to make a patch that is glued on the tubing. With a 14 or 15ga needle, punch a hole through the center of the folded square. Feed the silastic tubing partially into the needle. Slide the patch off the needle and onto the tubing at a point several cm from one end of the tubing. The exact distance of the patch from the end of the tubing is the estimated distance to the heart. This distance is 25 mm in 250 gm rats and 22 mm in 115 gm rats. See Fig. 2.1. Apply large beads of silastic adhesive between the tubing and the patch. Coat the junction completely with the adhesive. Allow the adhesive to cure 24 hours.

To modify needles used for insertion of the cannula into the vein: Bend a 1.5 inch 23 ga needle as shown in Fig. 2.2. Cut the needle from its base. File the base until it is smooth. Apply a small "sleeve" of epoxy to the needle as shown in Fig. 2.2. Do not place the needle in contact with any surface until the epoxy dries.

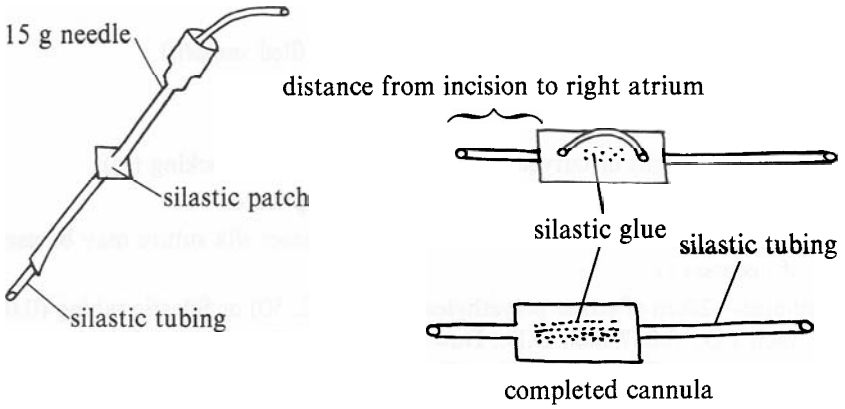

Figure 2.1. Preparation of an indwelling cannula. See text for description.

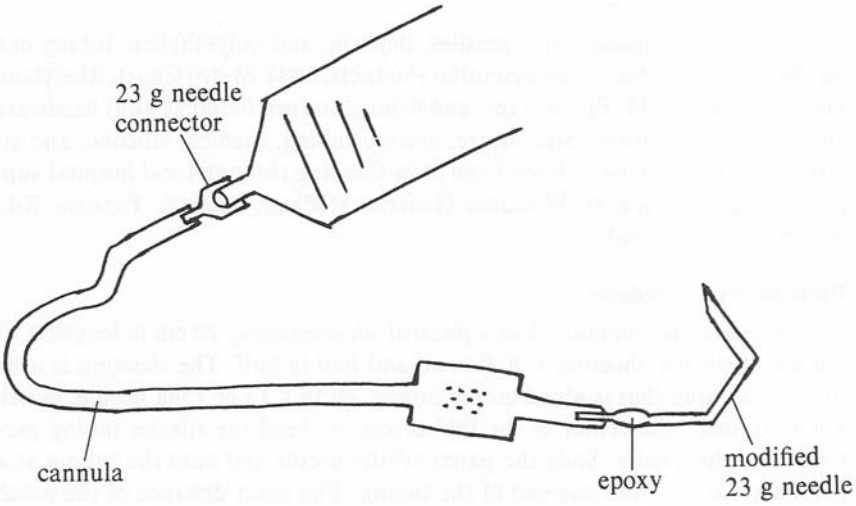

Figure 2.2. Modified needle used for insertion of a cannula into a blood vessel.

Although rats are unusually resistant to infection, sterilization of the cannulae and surgical instruments is recommended. All silastic materials can be autoclaved.

Anesthesia: Because the student must work directly over the animal's head, it is preferable to use an anesthetic which is injected rather than inhaled. The least expensive anesthetic of this type is probably urethane. Its disadvantages are a prolonged anesthesia, approximately 6 hours, and carcinogenic effects. Urethane should be handled only by the instructor. The recommended dose is

125 mg/100gm body wt., administered intraperitoneally (i.p). Urethane can be ordered from many chemical suppliers. Chloral hydrate and mixtures of chloral hydrate and sodium pentobarbital are reliable anesthetics for rats. The recommended dose of chloral hydrate is 300 to 400mg/kg body wt., administered i.p. Special Federal licenses are required to obtain these two compounds.

Surgical Procedures

Before beginning the surgery, fill a 1cc syringe with saline and attach it to a needle connector. Attach the connector to the long end of a cannula as shown in Fig. 2.2. Attach the short end of the cannula to the modified needle so the tip of the cannula touches the epoxy sleeve (Fig. 2.2). Flush the cannula with saline. Assemble all other items in the materials list.

Weigh a rat and administer the appropriate dose of anesthetic. Using animal hair clippers, shave the fur from the back of the neck, the thorax, and the junction of the thorax and right forelimb. Place the rat on its back on absorbent paper with the nose pointing toward the surgeon. Tape the forelimbs to the paper. Observe the pulse of the right carotid artery slightly anterior to the clavicle. With the scalpel, make an incision through the skin, beginning at the clavicle and extending anteriorally approximately 1 cm over the pulsating carotid. The incision will be slightly to the right of the midline. With forceps in each hand, tear the thin peritoneum horizontally beneath the skin incision. This should expose the jugular vein anterior to its disappearance beneath the muscle and the clavicle. If the rat is large (greater than 250gm), it may be necessary to tear through a layer of fat to expose the jugular. If the rat is small (less than 150gm), proceed with caution because the jugular will be immediately under the peritoneum. See Fig. 2.3, a and b.

During subsequent procedures, keep the incision moist with saline. Isolate the most anterior region of the exposed jugular vein by removing the tissue surrounding the vein. Slip one pair of forceps under the vein and grasp a piece of silk suture on the opposite side. Pull the suture under the vein so that the anterior end of the vein is isolated with a loose ligature as shown in Fig. 2.3b.

You will observe that the large vein has collapsed to a thin pink thread. Moisten the vein and do not touch it for a few minutes. The vein will dilate, but do not expect it to return to its original size.

Refer to Fig. 2.3 c as you complete the cannulation procedure. Holding the ligature with one hand, use the other hand to insert the modified needle into the posterior end of the jugular. Push the needle through both the vein and the muscle that is posterior to the vein. Pull the needle gently through the muscle until the cannula has emerged from the muscle. Remove the needle from the cannula. See Fig. 2.3 d.

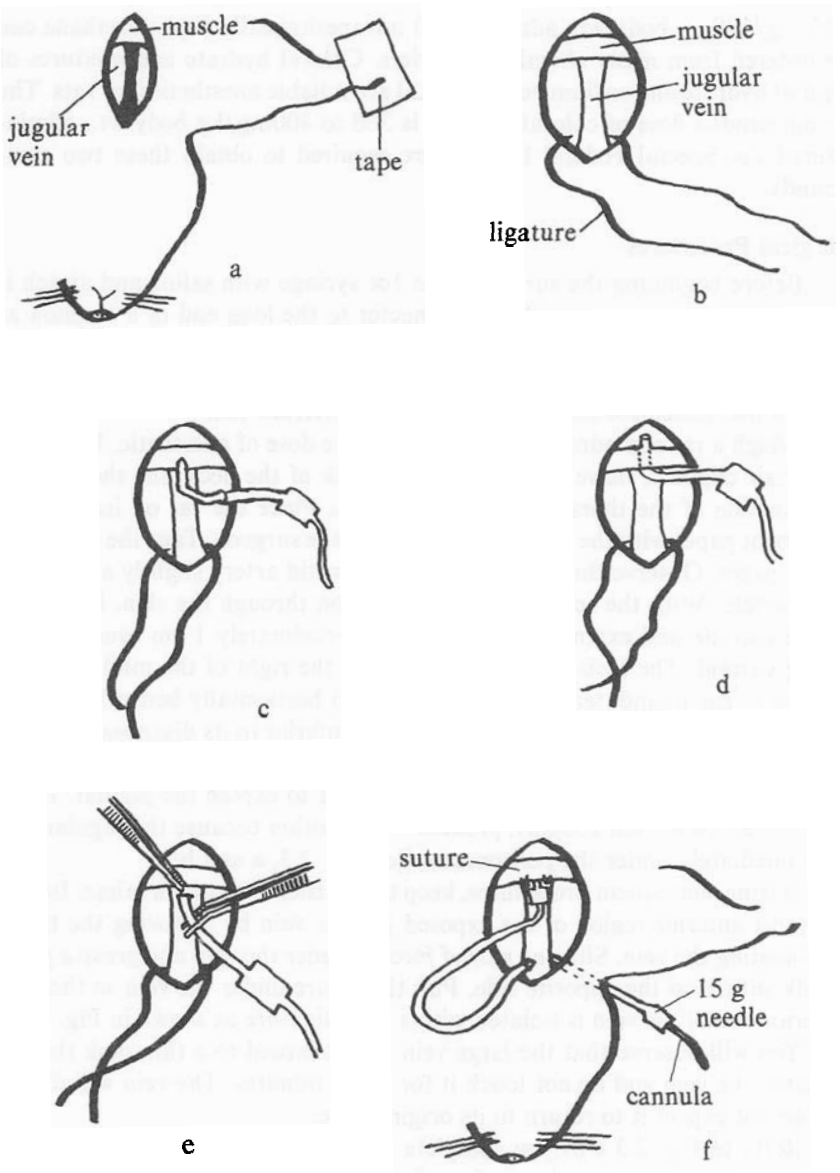

Figure 2.3. Flow diagram of a surgical procedure for implanting a chronic indwelling cannula into the right heart via the right jugular vein. a. Incision exposing jugular vein. b. Jugular vein isolated from surrounding tissues with a ligature. c. Use of a modified needle for insertion of a cannula into the jugular vein. d. Cannula inserted through the jugular and the overlying muscle. e. Use of forceps for retracting the vein wall, pulling the cannula backward, and then pushing it forward into the vein. f. Silastic sheeting sutured to the muscle and externalization of the cannula through a 15 ga needle.

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The last step of the cannulation requires a steady hand. Grasp the cannula with forceps near the point where it enters the jugular vein. With the other forceps, gently grasp the vein wall over the cannula. Pull the cannula backward until it is almost out of the vein, then gently slide it forward into the blood vessel. See Fig. 2.3 e. What if you accidently pull the cannula out of the vein? Use the ligature to occlude the flow of blood and attempt to reinsert the cannula into the hole in the vein. You will know when the cannula is within the blood vessel because 1) it will slide into the vessel easily and 2) you will be able to withdraw blood through it. Remember to flush the cannula with heparinized saline to prevent clotting within the cannula.

When the entire length of cannula distal to the patch has been inserted into the vein, you can remove the ligature from the anterior end of the jugular and suture the patch to the muscle. Two sutures are adequate. Pull the curved needle through both the muscle and the patch and tie a simple surgical knot. See Fig. 2.3 f.

Now you are ready to bring the cannula outside the rat's body. Remove the tape from the forelimbs and turn the rat on its belly. Insert a 15 ga needle under the skin on the back of the neck. Push the needle subcutaneously from the back of the neck until it emerges at the anterior end of the incision made previously. Remove the cannula from the needle connector and syringe and feed the cannula through the 15 ga needle until it emerges at the back of the neck. See Fig. 2.3 f. Remove the 15 ga needle from the rat and reconnect the cannula to the needle connector and syringe. Attempt to withdraw a blood sample through the cannula. You may have occluded blood flow by twisting or pinching the cannula; if so, adjust the cannula's position. Flush the cannula with saline. Close the inner tissues with 1–2 sutures. Close the incision with 1–3 wound clips, taking care not to pinch the cannula-patch with the clips.

The surgical procedures are finished at this time. To maintain the cannula for future experiments, flush it with approximately 0.15 ml of heparinized saline, occlude it by pinching, and cut it to a length of approximately 5 cm. Plug the cut end of the cannula with a piece of nylon fishing line, 30 # wt, 1.5 cm in length.

No special postoperative care of the cannulated rats is required, with the exception that they should be housed individually. Animals awaken from this procedure with no obvious signs of discomfort and resume eating and drinking within 6 to 12 hours. Terkel and Urbach (1974) found that basal levels of hormones were normal after the first postoperative day.

When all experiments have been completed and the cannulae are no longer useful, animals can be used in other experiments or killed by humane procedures (see McDonald et al. 1978 for methods of euthanasia).

References

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