Pharmacology Biochemistry & Behavior, Vol 16, pp 521–523, 1982 Printed in the U S A

Acute Venous Catheterization for Integrated Plasma Sample Collection in Monkey[1]

MARK P BREE, NANCY K MELLO, KATHY L HARVEY AND SUSAN A WEBB

Alcohol and Drug Abuse Research Center
McLean Hospital-Harvard Medical School, Belmont, MA 02178

Received 24 December 1981

BREE, M P, N K MELLO, K L HARVEY AND S A WEBB *Acute venous catheterization for integrated plasma sample collection in monkey* PHARMAC BIOCHEM BEHAV **16**(3) 521–523, 1982 —A method is described which permits acute percutaneous venous catheterization for continuous blood collection over a period of several hours This procedure can also be used for collection of repeated bolus samples without additional venipunctures Potential applications include collection of integrated plasma samples for neuroendocrine analysis of discrete plasma samples for pharmacokinetic studies

Acute venous catheterization Primates Blood sample collection Integrated plasma sampling

OVER the past year, the neuroendocrine effects of various drugs have been studied in this laboratory Our experimental design requires repeated studies of the same female monkey across four different phases of the menstrual cycle, over multiple cycles, to evaluate effects of alcohol and isocaloric control solutions on reproductive hormone levels

Since both gonadal steroids and pituitary gonadotrophins are secreted episodically, integrated plasma sampling procedures are essential to follow the episodic fluctuations in hormone activity [3,4] Integrated plasma sampling requires continuous collection of blood over a defined time period Each 20 or 30 minute sample reflects the true mean value of each hormone measured

Blood collection procedures which involve long term (weeks) catheterization and limb immobilization [2] are not appropriate for this purpose Moreover, we wanted to avoid possible complications (adhesions) involved in catheter implantation procedures which involved surgical exposure of the venous site [1] since repeated samples had to be collected from the same monkey over many months (8–16 months)

To meet this challenge, we have developed an acute procedure to obtain integrated plasma samples which involved percutaneous placement of a pediatric grade catheter in the saphenous vein The numerous problems which can plague repeated venipuncture, such as infective thrombosis, thrombophlebitis, and phlebothrombosis, have not occurred with this procedure Successful acute catheterizations have been performed on over 150 occasions in male and female rhesus monkeys with no adverse effects A minimum of 23 ml and a maximum of 35 ml of whole blood can be obtained safely over periods of 4 to 6 hours Catheterizations and blood withdrawal have been performed up to three times per week on the same animal This procedure is facilitated by the use of light sedation with ketamine hydrochloride (Ketaset) which eliminates problems such as respiratory and cardiac suppression often associated with anesthetics such as sodium pentobarbital, etc It is also useful to adapt the monkey to a restraining chair on several occasions before the first catheterization

METHOD

Acute venous catheterization was performed under light ketamine anesthesia (5–10 mg/kg IM) in 18–20 hour fasted animals This dose of ketamine has proven sufficient to maintain anesthesia for at least 20 minutes in monkeys weighing between 4 0 and 7 3 kg (Mean=5 45 kg) If additional anesthesia is required, more ketamine can be given in 10 mg increments at 5–10 minute intervals However, the entire catheterization procedure usually takes less than 20 minutes

Strict aseptic techniques were followed throughout the venipuncture procedure The monkey's leg was shaved and scrubbed with an antiseptic surgical prep solution A 19 ga needle which contained a 22 ga Deseret Radiopaque Intracath with wire stylet (20 3 cm) [Cat No 3166] was in-

[1]This research was supported by Grant No AA04368 from the National Institute on Alcohol Abuse and Alcoholism, ADAMHA

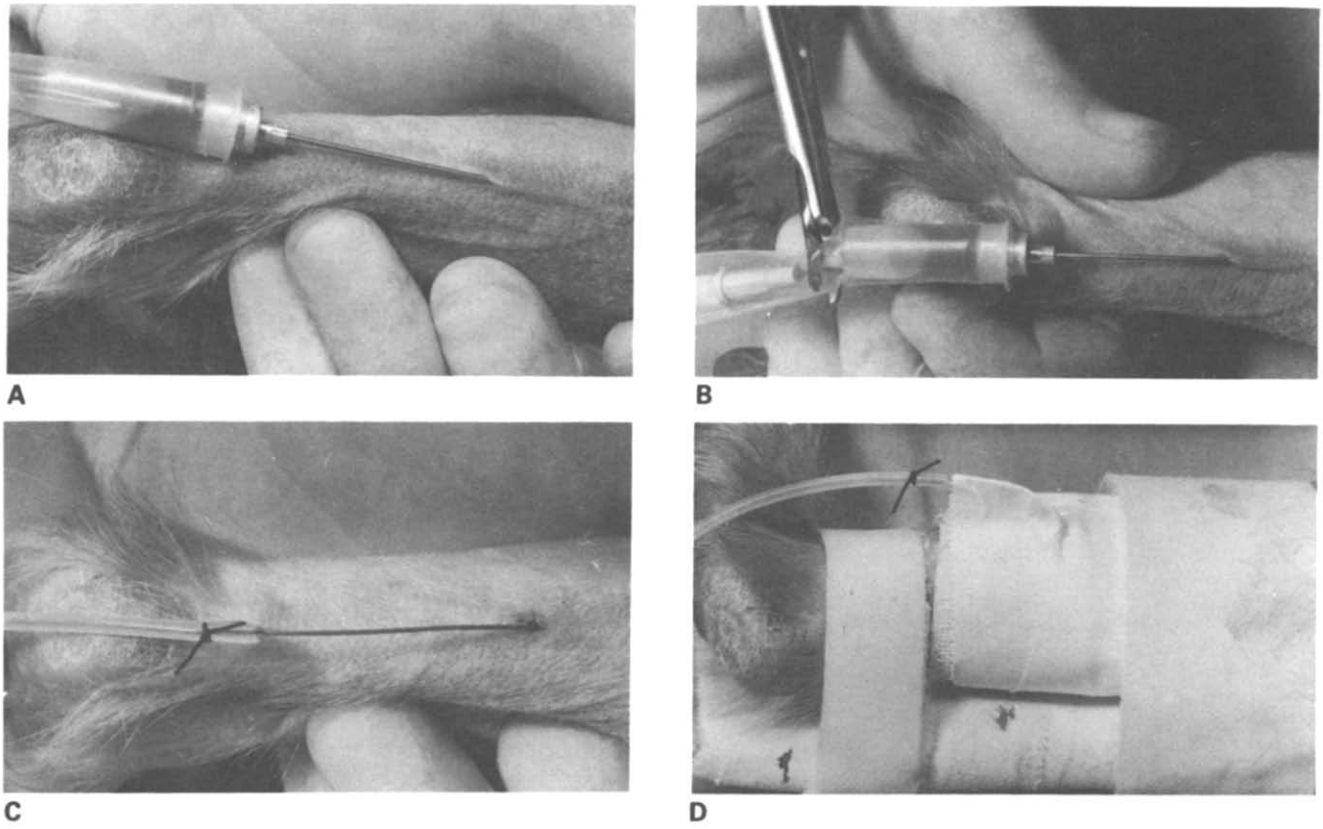

FIG 1 Acute venous catheterization procedure (A) Initial saphenous venipuncture with a Deseret Radiopaque Intracath (B) Separation of the intracath and stylet from the distal end of the plastic sleeve (C) Attachment of the intracath to the sterile silicon tubing (D) Final assembly of intracath and silicon tubing taped to leg with leg secured to Plexiglas splint

serted into the saphenous vein about 2 5 cm from the ankle (see Fig 1A) In the event the first venipuncture fails, a second venipuncture can be attempted above the initial puncture site After venipuncture, the intracath and stylet were fed through the 19 ga needle into the saphenous vein The ease with which the catheter and stylet enter the vein is a sensitive indicator of the success of the venipuncture The intracath stylet and the plastic sleeve that encases it were then cut just below the plastic introducer with a surgical wire cutter (see Fig 1B) The tip of the intracatheter was then dissected away from the stylet with a scalpel to facilitate removal of the stylet After the stylet was removed, the tip of the intracath was cut with a scalpel to provide a clean edge

Upon removal of the stylet from the intracath, there is usually some backflow of blood which indicates the correct positioning of the catheter in the vein The patency of the catheter is tested by flushing it with approximately 0 15 ml of a 1 20 heparin-saline solution, using a 1 cc syringe with a 26 ga needle If blood cannot be withdrawn, this indicates that the catheter is not in the lumen of the vein The intracath may thread easily along the venous sheath However, if there is no backflow of blood and blood cannot be withdrawn, the venipuncture procedure must be repeated

Once it has been determined that the catheter is in the vein, it is joined to a 76 2 cm length of sterile silicon tubing (i d =0 76 mm × o d =2 38 mm) (Patter Products, Beaverton, MI) presoaked in a heparin solution The catheter is secured in the tubing with a length of size 0 braided nylon suture (Fig 1C) The patency of the entire length of catheter is again tested with a saline solution, and the distal end of the catheter is clamped with a hemostat The intracatheter is secured in place with 2 5 cm elastic adhesive tape (Conform, Kendall Co , Boston, MA) The leg is immobilized with a simple plexiglas splint (5 cm × 1 cm × 22 cm) attached with velcro strips (Fig 1D) Once the catheter and hemostat are safely secured to the leg, the monkey is placed in a standard primate chair (BRS-Foringer)

A standard primate chair is used to maintain leg immobility during the blood exfusion procedure A 1 58 cm hole drilled in the bottom of the immobilizing splint is attached to the foot bar and the monkey's feet are secured to each foot bar with elastic adhesive tape Once the monkey is seated comfortably, the distal end of the catheter is attached to a 35 5 cm length of standard manifold tubing with a stainless steel connecting pin (Rainin Instrument Co , Inc , Woburn, MA) The tubing diameter is one determinant of the rate of blood flow For this application, tubing with an infusion and exfusion fluid flow rate of up to 0 90 ml/minute is preferable to smaller diameter tubing for maintaining constant blood exfusion with minimal clotting Occasional clots are readily cleared by detaching the intracath from the silicon tubing and flushing the tubing with normal saline Sterile technique should be maintained

For integrated plasma sample collections, blood is exfused with a Rainin Rabbit Miniature Peristaltic Pump (Rainin Instrument Co , Inc , Woburn, MA) The manifold

tubing is placed in the pump channel and the rate of flow is further adjusted by manipulating the pump speed For this application, a speed of 250 yields a total blood volume of 3 5 ml every 30 minutes The total blood volume collected per sample will vary as a function of the number of assays to be performed and the details of the particular assay procedure For discrete (bolus) blood sample collections, a sterile syringe with 26 ga needle is inserted into the intracath as often as required Intracath patency is maintained by periodic saline infusions

Blood samples are collected in 10 ml heparinized vacutainer tubes that are kept in chipped ice throughout the collection period Once the requisite amount of blood has been exfused, each sample is centrifuged at a speed of 10 k rpm for 10 minutes and appropriate aliquots of plasma are withdrawn and stored in polypropylene tubes and dry ice Samples are then frozen at $-20°$ until assayed

In applications involving intravenous drug administration, it is important to use a different vein to avoid contamination of the exfusion catheter

DISCUSSION

This acute venous catheterization procedure has been safe, effective and relatively simple to perform Over a 12 to 15 month period, we have completed an average of 28 catheterizations on the same monkey without any serious adverse effects If venipunctures are alternated between legs, it is possible to do two or three catheterizations on the same monkey within seven to ten days Upon return to the home cage, monkeys behave normally, i e , there is no indication of lethargy, compromised limb mobility, appetite reduction Moreover, there have been no instances of edema or infection associated with the venipuncture procedure If monkeys are properly adapted to the primate chair, they tolerate the procedure well and are reasonably placid under both drug and placebo conditions

This procedure permits repeated measures on the same animal under both continuous and discrete blood sampling conditions Although this report has emphasized blood collection procedures, the basic technique could also be used for short term drug infusions for studies of acute effects of drugs on a behavioral or biological variable

REFERENCES

1 Bedford, J A , A H Kibbe and M C Wilson Chronic intravenous dosing and blood collection in the unanesthetized monkey *Pharmac Biochem Behav* **6** 601–602, 1977

2 Conti, P A , T E Nolan and J Gehret Immobilization of a chronic intravenous catheter in the saphenous vein of African green and rhesus monkeys *Lab Anim Sci* **29** 234–236, 1979

3 Knobil, E The neuroendocrine control of the menstrual cycle *Recent Prog Hormone Res* **36** 53–88, 1980

4 Weitzman, E D Biologic rhythms and hormone secretion patterns In *Neuroendocrinology* edited by D T Krieger and J C Hughes Sunderland, MA Sinauer Associates, 1980, pp 85–92