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Chronic catheterization using vascular-access-port in rats: blood sampling with minimal stress for plasma catecholamine determination

C.S. Paulose and K. Dakshinamurti

Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, R3E 0W3 (Canada)

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Chronic catheterization is illustrated using vascular-access-port model SLA where the port is surgically placed subcutaneously on the back of the rat. The catheter is tunnelled to the neck and inserted into the jugular vein. Within 24 h rats showed normal blood pressure and blood samples were collected at intervals with minimal stress to the animals. A comparison of the plasma catecholamine of blood collected from vascular-access-ports with that obtained from decapitation indicates that there was minimal stress to the rats when blood was drawn through the vascular-access-port.

Introduction

The development of an easy access to the vascular system of small animals with minimal stress has become an important investigational method in many areas of research. Conscious animal studies, which necessitate the use of chronic catheterization, require the externalization of the catheter via a stab wound to connect it from the vasculature to the external monitoring device (Steffens, 1969; Flower, 1973; Brandstaetter and Terkel, 1977; Pass and Ondo, 1977; Burt et al., 1980; Bryan et al., 1983; Yoburn et al., 1984). Various other cannulation techniques have been developed to get reliable catecholamine levels in blood samples drawn in conscious rats (Buhler et al., 1978; Chiueh and Kopin, 1978; Carruba et al., 1981). Vascular-access-port (VAP) has been successfully

used in larger animals (Garner and Laks, 1985) where the whole device is implanted subcutaneously. VAP model SLA has been recently devised for use in small laboratory animals (Norfolk Medical Products, Skokie, IL, U.S.A.). In this study we present chronic catheterization of jugular vein in rats using VAP model SLA. This has been used to collect blood samples for plasma catecholamine determination in the conscious rat.

Materials and Methods

Animals

Sprague-Dawley rats weighing 200-300 g were used for catheterization. Animals were weighed and anesthetized using sodium pentobarbitone (50 mg/kg body weight i.p.).

Materials

Vascular-access-ports were purchased from Norfolk Medical Products, Skokie, IL, U.S.A.

Correspondence: K. Dakshinamurti, Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3E 0W3.

Norepinephrine, epinephrine and heparin were purchased from Sigma Chemicals, St. Louis, MO, U.S.A. Sodium pentobarbitone was purchased from Fischer Scientific, Fairlawn, NJ, U.S.A.

Method

Chronic jugular vein catheterization. The rat was anesthetized and hair removed from the back of the rat where the port was to be located. Skin was cleaned and sterilized using 70% alcohol. A 3 cm incision was made on the back of the rat and enough room was created in between skin and the body wall to locate the port. Prior to placement of the port over the body wall (Fig. 1) the port and the catheter were flushed with sterile saline and were filled with heparinized saline (1000 units/ml). The port was firmly secured to a body wall by sutures through the 4 reservoir base holes.

Another 2.5 cm incision was made on the right side of the neck to expose the jugular vein. The

catheter was tunnelled under the skin to the neck area. Excess of catheter length was cut. The skin on the back of the rat where the port was securely placed was sutured. The right external jugular vein was easily identified as it exits the thorax beneath the clavicle. Two 3-0 silk sutures were placed around the vein approximately 1 cm apart and the suture at distal end from the heart was tied to ligate the vein. A small venotomy was made anteriorly between the two sutures and the catheter was advanced in the vein towards the heart for a distance of approximately 2.5 cm (Fig. 2). The suture was tied around the catheter and vein. The blood flow through the catheter was checked by drawing blood by puncturing the septum of port placed on the back of the rat (Fig. 3). Huber point 24 gauge needles were used to puncture the rubber septum of the port. After making sure that the catheter was in the right place to sample blood, it was looped and the skin was sutured. The total

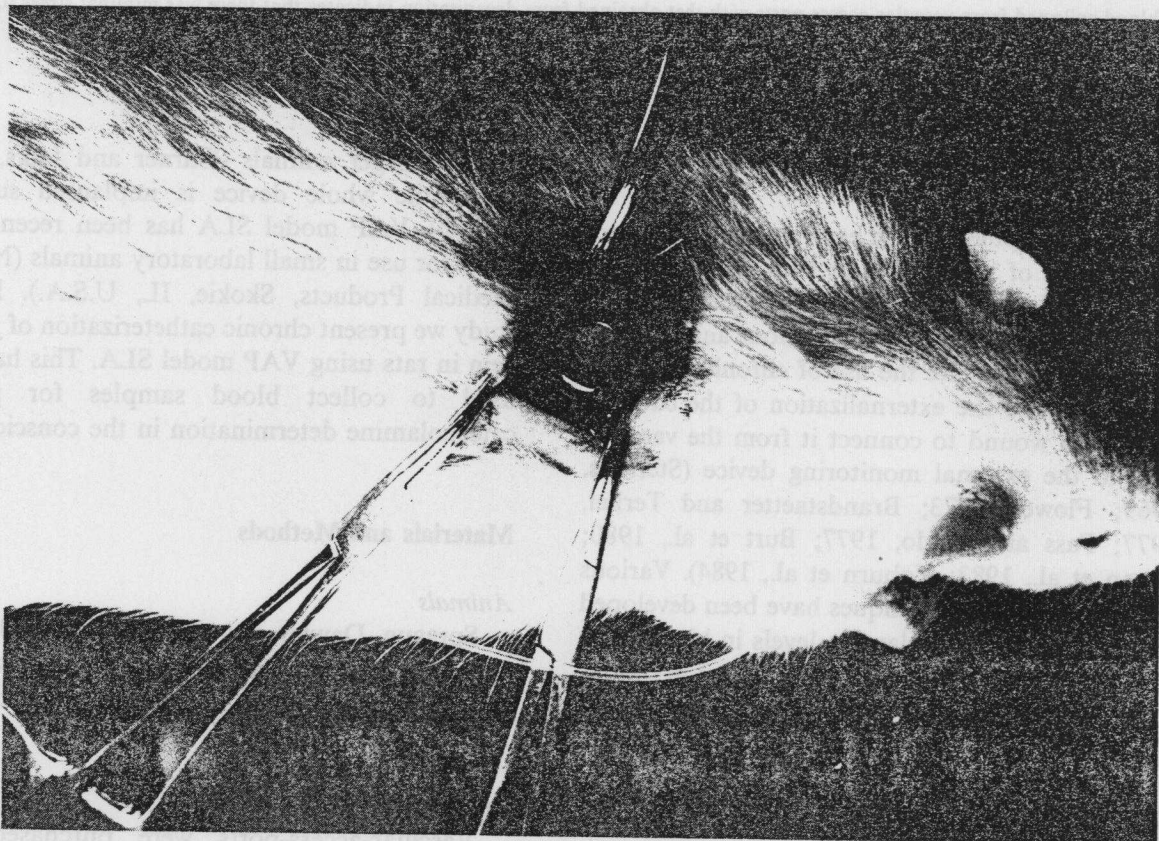


Fig. 1. The location and the size of the VAP sutured subcutaneously showing the catheter on the back of the rat.



Fig. 2. The catheter insertion to the jugular vein towards the atrium is shown. The suture knot to tie the catheter and vein together is clearly visible.

time taken for this procedure was 30–40 min.

Blood samples were drawn after 24 h, using a 5 ml syringe with a 21 gauge needle fitted to a polyethylene tubing (i.d. 0.030 in. \times o.d. 0.048 in. 24–30 in. long) which in turn was fixed to a Huber point 24 gauge needle. The needle and the tubing were filled with heparinized saline. Huber point 24 gauge needle was inserted into the rubber septum. About 15–30 min were allowed for the animal to recover from any stress of handling. The heparinized saline in the tube, the VAP and drops of blood immediately following this were discarded. Then 2 ml of blood were collected in a heparinized 5 ml syringe. The reservoir and the catheter were refilled with heparinized saline (1000 units/ml) after each sampling to avoid clotting of blood. Plasma was used for catecholamine determinations.

Extraction of catecholamines from plasma. 1.0 ml of plasma was transferred to a tube containing

1.0 ml of distilled water. 50 μ l of 5 mM sodium bisulfite was added and mixed. 250 μ l of 1 M Tris buffer pH 8.6 was added and mixed well. 20 mg of acid alumina was added. The contents of the tube were mixed using a rotator for 20 min. The supernatant was aspirated using a pasteur pipette. The alumina was washed twice with 2 ml of water containing 5 mM sodium bisulfite. To the final pellet of alumina 0.2 ml of 0.1 N perchloric acid was added and mixed for 15 min. The supernatant from this was used for HPLC determination of catecholamines.

HPLC separation conditions. 20 μ l of the extracted sample was injected into a Beckman HPLC apparatus with an Altex C18-1P reverse phase column (25 cm \times 4.6 mm i.d.; 5 μ m particle size; Beckman, Berkeley, CA, U.S.A.). The mobile phase consisted of 75 mM sodium phosphate monobasic, 1 mM sodium octylsulfate, 50 μ M EDTA and 11.5% acetonitrile. The buffer was adjusted to pH



Fig. 3. The syringe with 24 gauge Huber point needle is injected to the rubber septum of the port of the VAP to draw blood from the atrium. Catheter loop is filled with blood.

3.25 with phosphoric acid, filtered and degassed prior to use. A flow rate of 1 ml/min was used with a Beckman model 114 solvent delivery module. The catecholamines were identified by coulometric detection using an ESA model 5100 A detector with detector 1 set at a reduction potential of 0.05 V and detector 2 set at an oxidation potential of 0.04 V. A pre-injector guard cell was set at 0.45 V. The peaks were identified by relative retention times compared to standards and concentrations were determined by comparing peak areas using a Shimadzu integrator interfaced with the detector.

Blood pressure determination. Systolic blood pressure was measured by using tail cuff plethysmography in conscious rats.

Results

Twenty-four hours after catheterization the rats had completely recovered and continued to be

TABLE I

SYSTOLIC BLOOD PRESSURE BEFORE AND AFTER CHRONIC CATHETERIZATION IN THE ADULT RAT USING VASCULAR-ACCESS-PORT

Values are means \pm S.E.M. of 6 animals in each group. Values were not significantly different before and after catheterization. (Student's unpaired *t*-test)

Animal status	Blood pressure (mm Hg)
Before catheterization	119 \pm 3
24 hours after catheterization	114 \pm 4

alert and well for over two weeks. During this period blood samples were taken at two day intervals. Blood pressure of the catheterized rat returned to the normal value within 24 h (Table 1). The present method allows for the sampling of blood with minimal stress to the animals. Since the catheterization was done in the jugular vein there was no major trauma to the rats. Plasma catecholamine levels, determined 24 h after catheterization, are presented in Table II. The values are low and compare well with values reported using other catheterization techniques. This is in contrast to the several fold higher values for plasma catecholamines when blood was collected by decapitation.

Discussion

Plasma catecholamines have been studied in relation to blood pressure, anesthesia, ambient temperature, body temperature and certain biological characteristics e.g. age, sex, strain (Green and Miller, 1966; Cross and Houlihan, 1969; Michelfelder and Theye, 1971; Smith and Wollman, 1972; Reid et al., 1975; Roizen et al., 1974, 1976). Anesthetic agents like halothane, enflurane, methoxyflurane and barbiturates are known to cause cardiovascular depression. The concentrations of circulating catecholamines decrease or may not change. On the other hand ether anesthesia is accompanied by increased concentrations of circulating catecholamines (Gilman et al., 1985). Sequential blood sampling after decapitation showed an increasing catecholamine con-

TABLE II

PLASMA CATECHOLAMINE CONCENTRATIONS FROM THE PRESENTS STUDY COMPARED WITH LITERATURE DATA

Values are means \pm S.E.M. (Student's unpaired *t*-test). Numbers of animals per group are shown in parentheses.

Plasma catecholamines (pg/ml)			Site and method of blood collection	Reference
Norepinephrine	Epinephrine	Rat strain		
<i>Catheterization</i>				
518 \pm 48(8)	347 \pm 51(8)	Sprague-Dawley	Jugular vein	Present work
267 \pm 44	129 \pm 45	Sprague-Dawley	Carotid artery	Carruba et al. (1981)
567 \pm 65	350 \pm 157	Sprague-Dawley	Abdominal aorta	Popper et al. (1977)
477 \pm 22	372 \pm 19	Wistar-Kyoto (WKY) rats	Ventral tail artery	Chiueh and Kopin (1978)
<i>Decapitation</i>				
1 237 \pm 48(8)	2 953 \pm 101(8)	Sprague-Dawley	First 1.5 ml	Present work
3 800 \pm 200	8 100 \pm 760	Sprague-Dawley	First 3 ml	Popper et al. (1977)

centration in the collection period (Popper et al., 1977). Popper et al., (1977) have described an abdominal aortic catheterization technique for obtaining blood samples in a relatively unstressful condition in the rat. We tried carotid artery catheterization in many animals and measured systolic blood pressure by the tail cuff plethysmography 24 h later. In our experience the systolic blood pressures in such arterially catheterized rats were high (152 ± 8 mm Hg). Carotid artery catheterization causes a deep wound and will also block blood supply to brain whereas jugular vein catheterization is only associated with minor damage to the surrounding tissues. Abdominal artery catheterization cuts a major supply to the hind limbs. Long time abdominal aorta catheterization paralyzes the legs. In fact, abdominal aorta banding is a commonly used technique for producing hypertension and hypertrophy of the heart (Lund and Tomanek, 1978). The rats with the catheter in the jugular vein had much less trauma and showed normal systolic blood pressure and lower plasma catecholamines within 24 h of this procedure. These animals continued to show normal blood pressure and catecholamines levels for two weeks following the catheterization.

There are a number of advantages of the vascular access port system over the other techniques for drawing blood from conscious animals.

(1) Surgical implantation of VAP is simple and less time consuming.

(2) The implanted device is extremely well tolerated by the animals, and its small size, weight, and low profile enable its use in small laboratory animals.

(3) The subcutaneous location of the port minimizes the risk of catheter sepsis. Once the initial implantation incisions are healed, no protective dressings, bandages or jackets are required to prevent the animal from interfering with the cannulation system.

(4) The silicone rubber septum can withstand numerous punctures, the port is re-usable, making it a highly cost-effective tool. Regular port flushing and establishment of heparin lock is a painless routine that is well tolerated by the animal.

(5) Repeated blood sampling from the systemic circulation may be performed without undue stress on unsedated animals.

Thus chronic jugular vein catheterization using VAP model SLA is a useful technique to have access to the vascular system in conscious small laboratory animals.

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