Chronic Arterial and Venous Catheterization of Conscious, Unrestrained Rats

GÉZA FEJES-T6TH, ANIK6 NÁRAY-FEJES-T6TH, DIETER RATGE, AND JÜRGEN C. FRÖLICH

SUMMARY A method is described for implanting arterial and venous cannulas in rats that requires only minor surgery. Catheters are introduced into the abdominal aorta through the ventral tail artery and into the vena cava through a lateral tail vein. The wounds are covered with an acrylic cuff and the catheters are brought out through a stainless steel spiral connected to the cuff and then attached to top of a metabolism cage used to house the rat. This method makes possible continuous access to the catheters in undisturbed, mobile animals. Using this model we compared mean arterial pressure, heart rate, plasma renin activity, and plasma catecholamine levels in freely moving Long-Evans rats and in Brattleboro homozygous rats. (Hypertension 6: 926-930, 1984)

KEY WORDS • chronic vascular catheters • blood pressure • Brattleboro rat • plasma renin activity • plasma catecholamines

ALTHOUGH most of our present knowledge in animal physiology comes from experiments performed on anesthetized animals or from in vitro studies, the utility of these preparations is often curtailed when highly complex neural or hormonal regulatory mechanisms are investigated. Basal secretion rates and plasma levels of most hormones are drastically influenced by anesthesia and surgical trauma,1-3 and even simple handling of animals may have profound effects. The effect of general anesthesia on mean arterial pressure (MAP) and cardiovascular control mechanisms is well established,4,5 and even kidney function, which was generally regarded to be immune to these interventions, has recently been found to be markedly depressed during and following general anesthesia and surgical stress.6-9 Although methods have been developed to study larger laboratory animals under relatively untroubled conditions, such methods have not been generally adopted for the rat.

We describe a simple, chronic rat preparation that makes possible intravenous infusions, continuous monitoring of MAP and heart rate (HR), and repeated blood sampling in freely moving animals. Since vasopressin has been suggested to be involved in the regulation of blood pressure,10 we have also compared the resting MAP of the Brattleboro rat suffering from hereditary diabetes insipidus with the blood pressure of the normal Long-Evans rat from which the Brattleboro rat has been derived.

Materials and Methods

A slightly conic protecting cuff with a bulging middle portion was made from a rod of plexiglass on a lathe (Figure 1). A hole was bored into the middle portion of the cuff, and perpendicular to it a longitudinal slit was made. A 3 mm diameter stainless steel spiral, which had been previously stretched to 105% of its original length to render it more flexible, was glued into the cuff with epoxy. The first few centimeters of the spiral were fortified by surrounding it with a 4 mm diameter spiral. Two 55 cm long PE-10 tubings (Clay-Adams, Parsippany, New Jersey) were fed through the spiral, the proximal ends of the catheters were cut at a 90° angle, and the resulting edge was rounded off.

The protecting cuff and the catheters were sterilized with ethylene oxide at 22°C. Other materials and instruments were sterilized by soaking them in benzalkonium chloride (Zephirol, 1:750) solution and rinsing them with sterile isotonic saline before use.

Female Long-Evans or Brattleboro rats, 16 to 20 weeks of age, were kept in individual plastic metabolism cages (Nalgene, Rochester, New York) for 10 days prior to implantation of the catheters. The animals were anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and pentobarbital (15 mg/kg). Before surgery, the hair of the proximal part of the tail was clipped and a small part of the skin covering the ventral tail artery and tail vein was shaved. The skin was sterilized with ethylene oxide at 22°C. Other materials and instruments were sterilized by soaking them in benzalkonium chloride (Zephirol, 1:750) solution and rinsing them with sterile isotonic saline before use.

The tail artery and tail vein were cut. The tail artery was ligated with a 6.0 silk thread, a small amount of thrombin and some penicillin and
streptomycin were placed into the wound, and the wound was closed with an atraumatic needle with a 4.0 silk thread. One of the side veins of the tail was also exposed through an approximately 3 mm surgical incision and cannulated with the other PE-10 tubing, which was then advanced approximately 10 cm into the vena cava. In a few cases, advancing the catheter into the vena cava was somewhat difficult because the catheter got stuck after about 4 cm of advancing. However, in most cases this difficulty could have been overcome by slightly withdrawing the catheter and readvancing it after the animal’s position had been changed. After the second incision had been closed, the wounds were sprayed with neomycin powder, (Ne bacetin, BYK Gulden, Germany).

The protecting cuff (Figure 1) was positioned around the tail so that its middle portion covered the wounds while the proximal and distal parts were glued to the skin with an ethereal mastix solution. Thereafter the cuff was secured by a stainless steel wire, with special care taken not to compress the tail. The rat was placed back into the metabolism cage, and the spiral leading from the cuff containing the catheters was connected to the top of the cage through an adaptor with a bore diameter of 4.5 mm, and was secured against slipping back with a small wire. The catheters were filled with a minimal volume of heparin solution (500 U/ml) and closed with stainless steel wires (1 mm diameter). The catheters were flushed with a solution of heparin every day. All rats were allowed to recover from the operation and to get accustomed to the tail cuff for at least 4 days.

For continuous monitoring of MAP and HR, the arterial catheter was connected with the aid of a flow-through swivel to a strain gauge (Micron MP 15, H. Sachs Elektronik, March-Hugstetter, Germany) and an electromanometer (H. Sachs). The pulsatile signal from the electromanometer was used to drive a tachometer (Digi-Puls, MP 411, H. Sachs) that monitored HR. Blood pressure and HR were monitored continuously from 9 a.m. until 5 p.m. on the 5th and 6th postoperative days. The values recorded on each day were integrated by planimetry. Blood samples for the determination of plasma catecholamines and plasma renin activity were withdrawn between 11 a.m. and 2 p.m.

Results

Over 93% of the catheters remained open for at least 2 weeks after implantation. The average duration of catheter potency was 87 ± 13 days (n = 43, mean ± SE) in the case of the arterial catheter and 64 ± 18 days in case of the venous catheter. It should be noted, however, that as we acquired more experience with this technique, the period for which the catheter re-
mained open tended to increase. Indeed, currently we have a number of healthy rats in which the catheters are still functioning 5 to 9 months after implantation. As expected, MAP and HR in the conscious, freely moving rats showed a relatively high variability (Figure 2). In general, feeding, grooming, and locomotion caused a prompt rise in both MAP and HR, while drinking had no significant effect (Figure 3). The mean increase in MAP during feeding or grooming was 14.1 ± 2.0 (n = 7) and 10.9 ± 1.7 (n = 6) mm Hg, respectively while HR increased by 49.9 ± 12.2 bpm (n = 7) and 44.3 ± 10.7 bpm (n = 6) in Long-Evans rats. Interestingly, MAP and HR were not significantly altered when the animals fell asleep for brief periods. In addition to these rapid changes, significant slow shifts in MAP and HR were observed that could not be correlated with the animals' activity or behavior nor with day cycles. Patterns of the rapid and slow changes in MAP

FIGURE 3. Changes in mean arterial pressure and heart rate during various activities in Long-Evans rats (LE, top) and Brattleboro rats (DI, bottom).
and HR were similar in Long-Evans and in Brattleboro rats. The integrated values for MAP and HR from 9 a.m. until 5 p.m. are summarized in Table 1. As can be seen, there was no significant difference between Long-Evans and Brattleboro rats in either of these parameters.

The concentration of plasma catecholamines was also similar in the two groups of animals. Plasma renin activity, on the other hand, was significantly higher in Brattleboro rats (Table 1).

### Discussion

The profound effects of anesthesia and surgical stress on a number of physiologic functions are well established. Thus, to get meaningful results one has to use conscious, nonstressed, freely moving animals, but this may be difficult, especially in small laboratory animals such as the rat. Experimental procedures often involve repeated withdrawal of blood samples, continuous monitoring of blood pressure and HR, short-term intravenous infusions, and other maneuvers. There have been a number of attempts to overcome these difficulties, but experiments involving vascular catheterization in conscious rats are still not commonplace. In the most widely used "conscious" rat model, catheters were inserted under ether anesthesia, and after the animal was placed into a restraining cage, the experiments were performed in the ensuing 1 to 3 hours.

Although these animals might have regained consciousness, it is logical to assume that they were still under some degree of stress, which may have disturbed at least some parameters in addition to the anesthesia and surgical stress.

Chronic implantation of vascular catheters, therefore, is a more physiologic approach. However, chronic rat preparations are not necessarily devoid of some difficulties. For instance, cannulation of a common carotid artery may interfere with blood pressure regulation, while implantation of catheters into the femoral artery may lead to paralysis and gangrene of the corresponding hindleg in some strains of rats. Weeks and Jones circumvented these problems by implanting the arterial catheter directly into the abdominal aorta, but the disadvantage of their method was that it involved major surgery. To reduce the trauma that results from abdominal surgery, Chiueh and Kopin cannulated the tail artery, but they brought the catheter out on the back of the neck and failed to advance the tip of the cannula into the abdominal aorta; thus, catheter longevity in their preparation did not exceed 2 weeks. Almost invariably, the aforementioned techniques are conducted while the animal is immobilized in a restraining cage. Relatively few attempts have been made to perform experiments on freely moving animals.

The only major difference between our method described here and other available techniques is the way the catheters are exteriorized and protected. This difference, however, makes possible the continuous use of chronic arterial and venous catheters for several weeks or months in nonstressed, freely moving animals at the expense of very simple minor surgery. The catheters are exteriorized through the same wound made for implantation, and thus the need to tunnel them subcutaneously to the head or neck region is eliminated. Furthermore, this method does not hinder the normal activity of the rats and allows the collection of data from undisturbed rats in long-term projects. The rats become accustomed to the tail cuff in a matter of a few hours. The fact that the animals were, indeed, not in discomfort or under stress is demonstrated by the finding that plasma catecholamine concentrations were not in discomfort or under stress.

### Table 1.

<table>
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<tr>
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<th>Long-Evans rat</th>
<th>Brattleboro rat</th>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>101.8±2.15 (n=22)</td>
<td>100.7±1.95 (n=21)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>360.4±10.7 (n=22)</td>
<td>354.8±9.1 (n=21)</td>
</tr>
<tr>
<td>PRA (ng ANG l/ml/hr)</td>
<td>3.65±0.68 (n=13)</td>
<td>9.65±1.04 (n=7)</td>
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<tr>
<td>Plasma NE (pg/ml)</td>
<td>289±30 (n=13)</td>
<td>302±33 (n=7)</td>
</tr>
<tr>
<td>Plasma E (pg/ml)</td>
<td>154±21 (n=13)</td>
<td>169±39 (n=7)</td>
</tr>
<tr>
<td>Plasma dopa (pg/ml)</td>
<td>56±10 (n=13)</td>
<td>46±5.9 (n=7)</td>
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Values are means ± SE Statistical analysis of the data was performed by Student's unpaired t test. MAP = mean arterial pressure; HR = heart rate; PRA = plasma renin activity; NE = norepinephrine; E = epinephrine; dopa = dopamine; ANG = angiotensin; NS = not significant.
data and those published in references 15, 16, 25.) It is interesting to note that, in contrast to other studies, the MAP and HR in Long-Evans rats were not statistically significantly different from those measured in Brattleboro rats, which are unable to secrete vasopres-
sin. Nevertheless, it is not possible to exclude a role for vasopressin in the support of normal blood pressure, because, in accordance with previous observations, the plasma renin activity of Brattleboro rats was found to be significantly higher than in age-
matched Long-Evans rats. The difference may either represent a compensatory mechanism counter-balanc-
ing the absence of the pressor action of vasopressin or it may be a consequence of the volume contraction of Brattleboro rats. In contrast to a previous report, plasma catechol-
mine concentrations in the Long-Evans and Brattle-
boro rats in our present study were not significantly different. The reason for the discrepancy is not readily apparent, but it is likely to be related to the use of anesthesia and surgical stress in the aforementioned study.

We believe that the data reported here demonstrate the feasibility of our method and that other laboratories should be encouraged to adopt this simple method for studying truly conscious, unrestrained animals.

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