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Inexpensive Continuous-Infusion Swivel: Towards More Physiological Measurements

G. FRÜHBECK

Department of Physiology, School of Medicine, University of Navarra, Pamplona, Spain

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Summary

A cheap, simple and fast procedure of building an infusion swivel is described for both short- and long-term experiments in rats. To assemble the swivel, plastic laboratory syringe elements, needles and a three-way stopcock are used. The swivel avoids cannula-kinking and permits animals free movement inside the cage. In addition, the gadget presented is inexpensive enough to make it disposable. Further advantages of the self-made infusion swivel depend on its disposability, it is time-saving, simplifies blood sampling or administration of drugs and prevents contamination, when using radiolabelled products. The haemodynamics and metabolism of rats subjected to restraint stress or ether-anaesthetized rats significantly differ from those of conscious freely moving animals. Whereas restraint stress provoked a more pronounced increase of blood pressure and heart rate, ether anaesthesia induced a 19 % rise in serum glucose. Thus, the device described in this study provides more physiological experimental conditions.

Key words

Rat – Ether stress – Restraint stress – Infusion experiments – Disposable equipment

Introduction

Vascular cannulation in the rat is an important and useful experimental method (Coccheto and Bjornsson 1983, Tsukamoto *et al.* 1984). Many infusion experiments have to be conducted under light anaesthesia or in restrained animals. However, usual animal handling, including restraint, anaesthesia or blood sampling, easily produce stress and disrupt the physiological endocrine patterns of the rat (Mattheij and van Pijkeren 1977, Scott and Trick 1982). Swivels permit free movement of animals and overcome cannula collapse or kinking thereby ensuring continual flow. Commercial infusion swivels work perfectly, but are too expensive to be regarded as disposable material by many researchers. Therefore, to avoid fibrin clots or crystal precipitate obstruction immediate and careful cleaning is essential after the use. This paper describes the use of easily obtainable laboratory materials to build a cheap, disposable infusion swivel. Due to its disposability, this swivel reduces the upkeeping time required by other expensive models and, when administering radiolabelled solutions, eliminates the possible danger of subsequent radioactive contamination on the reuse.

Acute physiological stress responses have two functions. They organize the organism to cope behaviorally and physiologically with the challenge and, at the same time, they facilitate learning and memory processes which allow the animal to react more adequately to a similar stressor in the future (Koolhaas *et al.* 1993). Elevations of plasma hormones and catecholamines due to handling may diminish the relative differences in the control and experimental groups. For this reason, experiments should be designed to minimize stress, as much as possible (Sarlis 1991; Joint Working Group on Refinement 1993) and one should consider the question whether and/or to what extent the chosen housing and experimental conditions will affect the animals' state in terms of behavior, physiology and well-being. The present study was aimed to determine whether blood sampling without touching the rat yielded different cardiovascular and metabolic results as compared to anaesthetized or restrained animals.

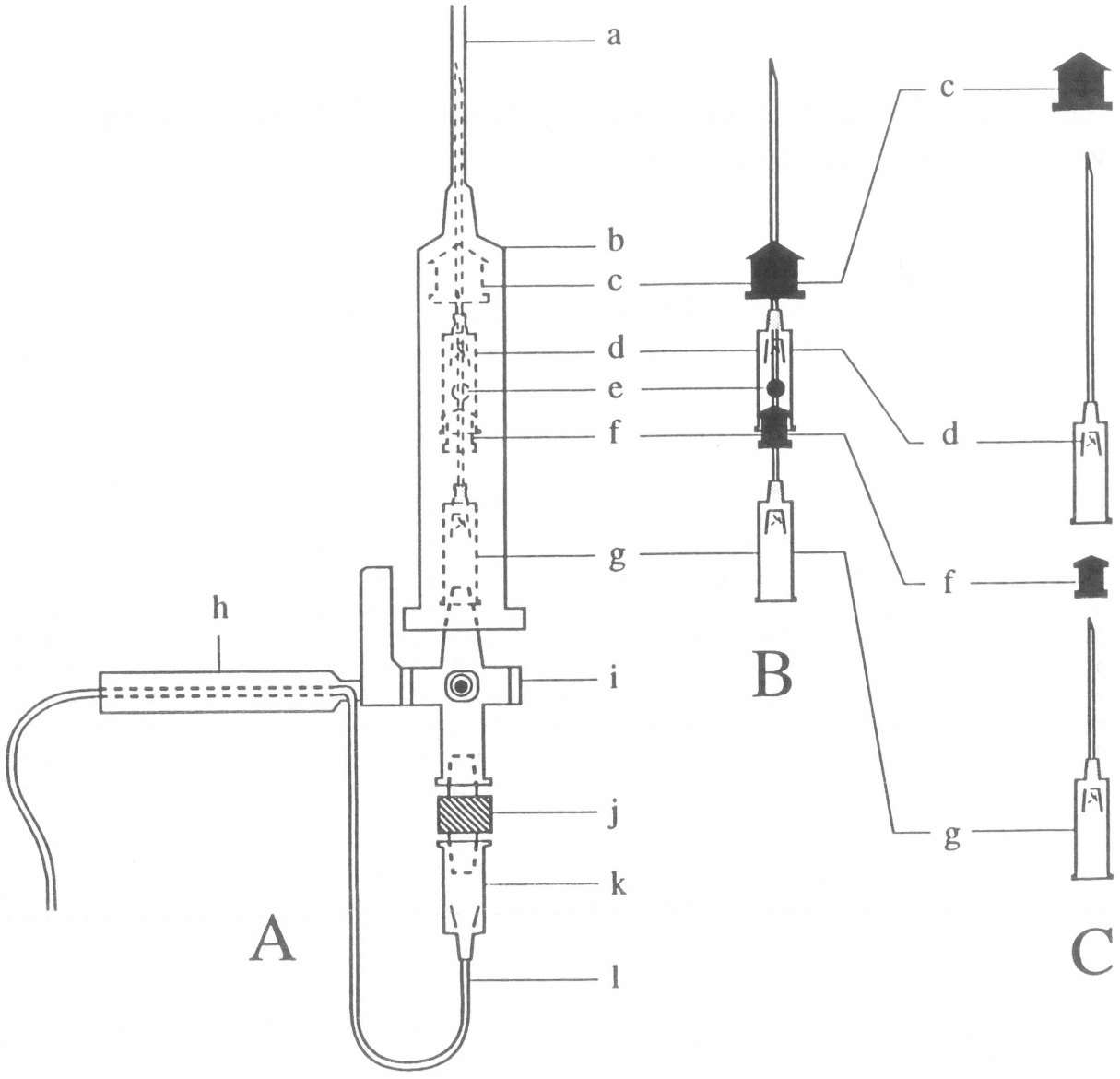

Fig. 1
Self-made infusion swivel. A – Fully constructed view, B – Close-up of the assembled parts before being introduced into the 3 ml plastic syringe barrel, C – Needles and rubber plungers before being mounted. Elements of the infusion swivel: *a* tubing to infusion pump, *b* 3 ml plastic syringe barrel, *c* rubber plunger from a 3 ml plastic syringe, *d* 1.3 x 40 mm disposable needle, *e* soldered joint, *f* rubber plunger from a 1 ml plastic syringe, *g* 0.8 x 40 mm disposable needle, *h* 1 ml plastic syringe barrel, *i* three-way stopcock, *j* male-male connector, *k* disposable stub adaptor, *l* tubing to animal

Material and Methods

Swivel assembling

First prepare the elements (*a-l*) used to assemble the infusion swivel as depicted in Fig. 1. Secondly, scar the shaft of a 0.8 x 40 mm needle (*g*) at 9 and 11 mm from the joint with the plastic hub. Then, smooth the surface of the marked needle section with a file to prepare the area for later soldering. Next, introduce this needle through the centre of a rubber plunger from a 1 ml syringe (*f*) as shown in Fig. 1C. Deposit a solder pearl (*e*) on the prepared zone of the needle. Allow the solder to solidify.

Afterwards, push a 1.3 x 40 mm needle (*d*) through the rubber plunger of a 3 ml plastic syringe (*c*).

Slide the needle right through the centre of the plunger, slipping it into the plastic hub. Insert the tip of the prepared 0.8 x 40 mm needle through the open hub of the 1.3 x 40 mm needle (*d*) and advance it as far as possible. Introduce the male end of a three-way stopcock (*i*) into the hub of the small needle (*g*).

Introduce, as shown in Fig. 1A, the assembled parts (Fig. 1B) into the barrel of a 3 ml syringe (*b*). Join a male-male connector (*j*) to the three-way stopcock (*i*) and to a stub adaptor (*k*).

Cut the tip of a 1 ml plastic syringe barrel (*h*) with a red-hot scalpel blade. Then, with a red-hot needle produce a hole in the same syringe barrel to fit the tubing coming from the animal (*l*). In the centre of the three-way stopcock handle deposit a drop of acrylic glue and insert the tip of the 1 ml syringe barrel. This barrel works as a lever arm decreasing the momentum of torque needed to move the swivel. Adapt the swivel on a weighted ring stand with a biuret clamp approximately 25 cm above the cage.

Obviously, for the successful outcome of long-term infusions surgical skills are essential to position and fix the cannula (Van Dongen *et al.* 1990, Joint Working Group on Refinement 1993), but this is not within the range of the present communication.

Animals

Conventional male outbred adult Wistar rats (breeding centre of the University of Navarra) were 7 weeks of age (mean body weight 238 g) at the beginning of the study. Animals had free access to daily renewed tap water, and were fed *ad libitum* commercially pelleted rations (rat and mouse standard diet B & K Universal). In the animal room there was controlled temperature (20±2 °C), relative humidity (50±10 %), ventilation (at least 15 air changes per hour) and artificial light-dark cycle (light from 08.30–20.30 h).

Experimental design

Rats were randomly assigned to three different experimental groups of 10 animals each. Conscious, unstressed, freely moving animals, caged in pairs in plastic boxes 15 x 50 x 25 cm in size (Panlab, Spain) rather than singly housed (as we are dealing with social animals) served as the control group. Control animals remained in their cages throughout the experimental procedure and were not disturbed during recording of the blood pressure nor by blood sample withdrawal. Restraint stress was imposed to the second group by immobilizing the rats for 5 min in a tubular plexiglas cylinder (20 cm long, 5 cm internal diameter). Anaesthesia stress was induced by exposing the animals of the third experimental group to ether vapors for 5 min as described by Jörgensen *et al.* (1992).

Surgical procedures

Femoral venous cannulae [5 cm of PE-10 tubing (0.279 mm ID x 0.610 OD and 3 cm length) fused to PE-50 tubing (0.580 mm ID x 0.965 mm OD) at the site of cannulation] were surgically implanted by a method similar to that of Carnes *et al.* (1989). Besides this, the right femoral artery was also catheterized. In the control group the free ends of the cannulae were tunneled subcutaneously, exteriorized between the scapulae, passed outside the cage through a stainless steel coil attached to the self-made swivel and sealed with removable pins. This procedure allows the rats to move without restriction inside the cage. The dead space of the cannulae (0.60 ml) was filled with heparin (1000 IU/ml) and flushed twice daily with 20 IU/ml heparinized saline. All operations were performed under sodium pentobarbital anaesthesia (50 mg/kg i.p.) and aseptic conditions. Surgical incisions were infiltrated with 2 % lidocaine jelly, sutured and swabbed with betadine solution. The animals were given 0.1 ml penicillin G (300 000 U/ml, C. E. P. A., Madrid, Spain) and allowed to recover for at least 7 days.

Data acquisition

The catheter of the right femoral artery was connected to a pressure transducer (Statham AA). The heart rate was counted from the pulses on the blood pressure recording. Taking into account that blood glucose was shown to change significantly 3–8 min after exposing the rat to the stress situation and that the heart rate reaches a peak 5 min after the beginning of experiment (Gärtner *et al.* 1980), blood pressure, heart rate and blood glucose data were recorded 5 min after beginning of the stress procedure and after a 12 h overnight fasting period.

Blood sampling and assay

Blood was collected from the venous cannulae following a similar method to that described by Tsukamoto *et al.* (1984). The withdrawn blood was then transferred into glass serum separation tubes, allowed to clot at room temperature (22 °C) for 15 min, and centrifuged (1200 x *g*) for 10 min. Samples were stored at −20 °C until assayed. Glucose levels were estimated because it had been demonstrated that they are good indices of the intensity of stress experienced by rats (Armario *et al.* 1986, 1991). Glucose was determined by the glucose-oxidase method using a commercial kit (Boehringer, Mannheim).

Statistics

Analyses were performed using the StatView 4.01 Non-FPU (Abacus Concepts, Inc. 1992-93) statistical package for Apple Macintosh computers. Data were analyzed by one-way analysis of variance (ANOVA), followed by Fisher's PLSD (least significant difference) pairwise comparison for identifying significant differences between pairs. Values reported are means ± S.E.M. $P<0.05$ was considered to indicate statistical significance.

Results

The dead space of the device was 0.60 ml. The self-made swivels held up to 40 kPa without leaks. Pressure values of 60 kPa were tested, but the swivels did not always resist.

All animals moved freely within the cage without any apparent disturbance. Swivels remained functional throughout the experimental period. Successful blood sampling in unanaesthetized and unrestrained rats was achieved.

Table 1 summarizes blood pressure, heart rate and serum glucose levels of the different experimental groups. First of all, it was found that stress induced either by restraint or anaesthesia significantly increased blood pressure as compared to the control group. Both systolic and diastolic pressures behave in the same manner. Highest pressure levels were observed in the restraint group.

As regard to the heart rate, it is noteworthy to point out that greater difference was found between the restraint and anaesthetized groups than between control and anaesthetized rats.

However, it is of interest to note that blood glucose was more markedly increased in the anaesthetized group as compared to both the restraint and control rats.

Table 1
Influence of restrain and ether anaesthesia on blood pressure, heart rate and serum glucose in male Wistar rats

Experimental groups Parameter	Controls	Restraint stress	Ether anaesthesia
Systolic pressure (kPa) (mm Hg)	18.96 ± 0.57 142	22.81 ± 0.56** 171	21.18 ± 0.35**# 159
Diastolic pressure (kPa) (mm Hg)	10.93 ± 0.29 82	13.85 ± 0.39** 104	12.86 ± 0.23**# 96
Heart rate (beats/min)	329 ± 3	436 ± 3**	345 ± 4*##
Blood glucose (mmol/l)	6.64 ± 0.03	7.16 ± 0.05**	7.87 ± 0.12**##

*Data are expressed as means ± S.E.M (n = 10). Significant differences: * $p<0.05$, ** $p<0.01$ compared to controls; # $p<0.05$, ## $p<0.01$ compared to restraint stress.*

Discussion

The conclusion drawn in this study is that haemodynamics and metabolism of either restraint stress or ether-anaesthetized rats significantly differ from those of conscious freely moving animals.

The raised blood glucose level in the anaesthetized group correlates with findings made in both in rats and humans (Scott and Trick 1982, Greene 1974). These researchers found that insulin values in ether-treated subjects doubled in response to the anaesthetic-induced hyperglycaemia.

However, this study was not able to reproduce the 9 % reduction in arterial pressure observed by Walsh *et al.* (1977). When looking closer at the pressure values, stress provoked an evident increase in both systolic and diastolic values, but pulse pressure hardly changed in all three experimental groups [about 8 kPa (60 mmHg)]. Maybe ether anaesthesia induces a biphasic response consisting of an initial blood pressure and heart rate increase (Morton 1990), due to the release of catecholamines (Pfeffer and Frohlich 1973), followed by a reduction of total peripheral resistance due to a direct vasodilator effect of ether on the arteriolar vasculature (Smith and

Hutchins 1980). Data acquisition in this study was accomplished 5 min after beginning of the stressful stimulus application, therefore, it only concerns the reactions in the first phase.

Obtained heart rate data are relatively similar to those reported by Gärtner *et al.* (1980) and are probably due to stimulation of the sympathetic nervous system. This is in contrast to the findings of Tadepelli *et al.* (1974) who found that ether anaesthesia did not alter the heart rate in either Wistar-Kyoto or spontaneously hypertensive rats. This apparent contradiction may be attributed to the fact that anaesthetic agents may have differential effects depending on the rat strain being investigated (Hall *et al.* 1976).

Implantable telemetry for monitoring blood pressure and other parameters provides a number of benefits including stress elimination caused by handling, restraint and anaesthesia (Brockway and Hassler 1993). Nevertheless, a potential drawback of telemetry systems is that drug administration and blood withdrawal can not be accomplished without handling the animal. The use of indwelling venous cannulae and repeated sampling from the same animal offers advantages for endocrine studies. In this particular aspect, swivel and tether systems may offer an advantage. The device described warrants free movement of rats within the cage and provides, in the interests of science and animal welfare, physiological circumstances for infusion experiments.

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Reprint Requests
G. Frühbeck, Department of Physiology, School of Medicine, University of Navarra, 31080 Pamplona, Spain.