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A novel, double intra-carotid cannulation technique to study the effect of central nutrient sensing on glucose metabolism in the rat☆

M. Rijnsburger[a,1], L. Eggels[a,1], J. Castel[b], C. Cruciani-Guglielmacci[b], M.T. Ackermans[c], S. Luquet[b], S.E. la Fleur[a,*]

[a] Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[b] Unit of Functional and Adaptive Biology, Paris Diderot University-Paris 7, Paris, France
[c] Department of Clinical Chemistry, Laboratory of Endocrinology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

HIGHLIGHTS

- We developed a method to infuse blood-borne substrates and concomitantly assess glucose kinetics.
- This route of central infusion is more physiological than the often used ICV infusion.
- We show that this technique does not affect corticosterone levels and energy expenditure in rats.

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ABSTRACT

Background: The hypothalamus plays a key role in central nutrient sensing and glucose homeostasis. Due to its position next to the third ventricle, intracerebroventricular (ICV) injections or osmotic minipumps are widely applied techniques in studying effects of hormones and other molecules on the hypothalamus and glucose metabolism.
New methods: The intracarotid catheter technique in which a catheter is placed in the carotid artery, pointing towards the brain, provides a physiological route to centrally infuse blood-borne molecules in an undisturbed animal. To measure effects of central interventions on peripheral glucose metabolism, endogenous glucose production (EGP) and insulin sensitivity can be measured using a stable isotope technique. To combine both techniques, it is necessary to combine different catheters. We here describe a novel cannulation technique for the carotid artery, enabling stress-free infusions towards the brain and blood sampling from the carotid artery concomitantly, and infuse a stable isotope via the jugular vein.
Results: We showed accurate EGP measurements when intracarotically infusing saline towards the brain. The stress-hormone corticosterone, as well as energy expenditure, did not alter upon central infusion.
Comparison existing method(s): ICV infusions bypass the blood-brain-barrier (BBB) and are thus a less physiological approach when studying central effects of blood-borne factors. Furthermore, ICV injections can elicit a stress response which can interfere with outcomes of glucose metabolism. We described a stress-free, physiological method to study effects of central infusions on peripheral parameters.
Conclusions: This technique provides new opportunities for studying central effects of, for instance, hormones and nutrients, on glucose metabolism.

1. Introduction

Blood-borne molecules like hormones and nutrients can cross the blood brain barrier (BBB) to affect central sites to influence behavior and metabolism. To study these central effects, many use cannulae implanted into different brain ventricles and infuse hormones, nutrients or drugs via intracerebroventricular injections (ICV) or osmotic minipumps. However, with this direct delivery in one of the ventricles, BBB is bypassed and thus infusions do

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* Corresponding author at: Department of Endocrinology & Metabolism Academic Medical Center, University of, Amsterdam, Meibergdreef 9, F2-131-2, 1105 AZ Amsterdam, The Netherlands.
E-mail address: s.e.lafleur@amc.uva.nl (S.E. la Fleur).
[1] These authors contributed equally.

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not reflect the physiological route by which blood-borne factors are affecting central sites. The ICV infusions however are used frequently because, for example, certain drugs are unable to cross the BBB (DeVos and Miller, 2013; Grathwohl and Jucker, 2013) and surgery to implant ICV cannulae and osmotic minipumps is relatively quick. Also when studying the central effect of metabolic hormones and substrates such insulin, leptin and glucose, this technique is widely accepted and applied (Muta et al., 2015; Air et al., 2002a; Air et al., 2002b).

To administer molecules/substrates more physiologically to the brain, it is possible to place an ascending catheter in the carotid artery, pointing towards the brain. Infusions via this route have proven effective in the field of central nutrient sensing (Migrenne et al., 2011; Moulle et al., 2013), and infused molecules, for instance glucose, have been shown to reach hypothalamic structures bilaterally (Dunn-Meynell et al., 1997).

As mentioned above, hormones and nutrients affect the brain to regulate feeding behavior and metabolism. Specifically, the hypothalamus plays an important role in nutrient sensing and is involved in regulating glucose metabolism. It contains glucose responsive neurons which either increase or decrease their firing rate, upon changes in blood glucose levels (Routh, 2010). In addition it has been shown that hypothalamic neurons are also responsive to fatty acids (Wang et al., 2006). Consequently to the modified activity of these neurons, glucose homeostasis is maintained. This can be established either by altering glucose uptake by peripheral tissue under the control of insulin, or by affecting endogenous glucose production (EGP). To measure the effects of central interventions on glucose homeostasis, EGP can be measured using stable isotopes. In this procedure, [6,6-2H2]-glucose is infused via a jugular vein catheter and blood samples are drawn via the carotid artery, where after isotope enrichment is measured by gas chromatography-mass spectrometry (GCMS) (Ackermans et al., 2001) and EGP calculated using Steele equations (Steele, 1959). In addition, during a hyperinsulinemic euglycemic clamp, an insulin bolus is infused through the jugular vein and the subsequent amount of exogenous unlabelled glucose that needs to be infused via the jugular vein to maintain euglycemia, is a measure for insulin sensitivity (Hughey et al., 2011). The method with stable isotopes to measure glucose metabolism is extensively validated using these descending catheters in jugular vein and carotid artery that allow continuous infusion and withdrawal of blood at the same time. It is possible to draw blood from other sides than the artery, for instance via tail incision, but to be able to perform these experiments in a freely moving and undisturbed animal, it is necessary to combine the carotid (ascending) catheter towards the brain with the (descending) catheter towards the heart to draw blood samples, and, infuse a stable isotope, insulin and/or glucose via the jugular vein catheter. We here describe how we have combined existing cannulation techniques and developed a never before shown ascending and descending catheter at the same location in the carotid artery making it possible to measure insulin sensitivity with stable isotopes at the same time as continuous infusions towards the brain. Moreover, we were able to conduct the experiment using this technique, in airtight controlled metabolic cages and measure energy expenditure as well, without eliciting a stress response.

2. Materials and method

2.1. Animals

Male Wistar rats (250–275gr on arrival, Harlan, Belgium) were housed five per cage in a temperature (±21 °C) and light controlled room (lights on: 7 am, lights off: 7 pm). They received an acclimatization period of 7 days. All animals had ad libitum access to laboratory chow (SAFE, Augy, France) and tap water. Animals were housed individually (35 × 25 × 25 cm plastic cages) after surgery.

The experimental protocol was approved by the institutional animal care and use committee of the Paris Diderot University (CEEA40), under the agreement # CEB-20-2015.

2.2. Catheters

Catheters were made of 10 cm pieces of silicone tubing (0.6 × 1.2 mm, Rubber BV, the Netherlands). Silicone glue was used as an anchor ring at 4.2 cm for the jugular, 1 cm for the carotid towards the brain (hereafter called ascending carotid) and 1.2 cm for the carotid towards the heart (descending carotid). At the end of the catheter two small holes were made with a blunted 20G (gauge) needle to prevent a vacuum during blood sampling and catheters were stored in 70% ethanol and rinsed with saline before surgery. Lengths were based on male rats weighing 275–300 g.

2.3. Surgery

Rats underwent surgery under anesthesia induced with an i.p. injection of 80 mg/kg Ketamin (Eurovet Animal Health, the Netherlands), 8 mg/kg Xylazin (Bayer Health Care, the Netherlands) and 0.1 mg/kg Atropin (Teva Pharmachemie, the Netherlands). To maintain anesthesia during surgery, we first injected 1/3 of the mix that was used to induce anesthesia. Thereafter, only when needed, ketamin was injected to maintain anesthesia. The animals were put on a heat mat during the surgery.

Insertion of the right jugular vein and tunneling of all three catheters to the head was performed according to the method described in detail in (Steffens, 1969). Hair on top of the head and in the neck was removed. A cut of 2 cm was made on top of the head using a surgical scalpel. The periosteum was anaesthetized locally using lidocaine and gently pushed aside. Four small holes were drilled using a dental drill in a square leaving enough space in between the two in front and in between the two in the back to fit the cannulas in between. Screws (1.2 × 3 mm, Fabory, the Netherlands) were inserted. We used four screws to ensure firm attachment of the cement, since a bigger cement is needed to embed the three cannula's and the metal connector.

The right jugular vein was cannulated according to the method of Steffens (Fig. 1 and (Steffens, 1969)). After externalization of the catheter on the head, a 90° bend and blunted 20G needle was pushed in the end of the catheter and the catheter was checked and flushed; first some blood was withdrawn and saline was pushed back to be sure the catheter was clean. Thereafter it was filled with 0.05 mL PVP solution (0.9 g PVP, polyvinylpyrrolidone K25 [81400], Sigma; 0.3 mL heparin, 5000IE; 0.7 mL amoxicillin, solved overnight or 2 h in 37 °C) and closed with a cap made of a piece of cauterized flexible tubing (0.6 mm inside diameter (ID), Technilab instruments, the Netherlands).

For the carotid catheters (Fig. 1B), an opening of about 1 cm was cut about 0.3 cm left of the trachea, and the left carotid artery was dissected and gently separated from the Vagus nerve using two small forceps, resulting in a clean part of the carotid artery of about 1.5 cm. A surgical thread (4-0 Mersilene fiber suture, Ethicon, USA) was put underneath the carotid and cut in half, on one side a loose knot was made while the other closed the carotid in the middle. For both cannulations a new thread was used and the second thread was knotted one on top of the first one to maximize free space and create the possibility to stretch the artery.

To insert the descending catheter, the artery was temporarily closed towards the heart with a Micro serrefine (FST, Germany) in such a way that a small space of about 0.5 cm was left to insert the catheter. The artery was gently stretched/put on tension with a bulldog serrefine (FST, Germany) hanging at the end of the surgical