Journal of Pharmacological and Toxicological Methods 86 (2017) 67–75

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

How to

Development of microdialysis methodology for interstitial insulin measurement in rodents

Ann E. Griffin[a,1], Ruth Macdonald[b,2], Anton J.M. Wagenmakers[c,3], Janice M. Marshall[a,*], Simon M. Poucher[b,4]

[a] School of Clinical and Experimental Medicine, The Medical School, University of Birmingham, Birmingham B15 2TT, United Kingdom
[b] Cardiovascular and Gastro-intestinal Research Department, AstraZeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, United Kingdom
[c] School of Sport and Exercise Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

ARTICLE INFO

Keywords:
Methods
Microdialysis
Insulin
Interstitial fluid
Zucker rat
Hyperinsulinaemic-euglycaemic clamp
External reference technique

ABSTRACT

Introduction: Accurate assessment of muscle insulin sensitivity requires measurement of insulin concentration in interstitial fluid (ISF), but has proved difficult. We aimed to optimise measurement of ISF insulin concentrations in rat muscles *in vivo* using microdialysis.

Methods: Factorial experimental design experiments were performed *in vitro* to determine optimal conditions for insulin recovery with microdialysis probes. These conditions were tested *in vivo*, adjusted appropriately and used in lean and obese Zucker rats to compare ISF insulin concentrations basally and during hyperinsulinaemic-euglycaemic (HE) clamp.

Results: Optimal conditions *in vivo* were: a 100 kDa microdialysis probe inserted in muscle, perfused with 1% BSA, 1.5 mM glucose in 0.9% sodium chloride at 1 µl/min. Samples were collected into siliconised glass microvials. As a reference for insulin, we established a protocol of inulin infusion, beginning at − 80 min and reaching equilibrium within 60 min. HE clamp, beginning at 0 min, increased ISF insulin concentration from 122 ± 56 basally to 429 ± 180 pmol/l ($P < 0.05$) in lean rats and from 643 ± 165 to 1087 ± 243 pmol/l ($P = 0.07$) in obese rats; ISF insulin concentrations were significantly higher throughout in obese rats. The difference between ISF and plasma insulin concentration (ISF:plasma ratio) was substantially higher in obese rats, but fell to similar values in obese and lean rats during HE clamp.

Discussion: Optimising insulin recovery with microdialysis allowed accurate measurement of basal ISF insulin in muscle of lean and obese Zucker rats and indicates insulin transport across capillaries is impaired in obese rats, basally and during hyperinsulinaemia.

1. Introduction

The prevalence of obesity and diabetes is increasing worldwide. Thus, there is an associated need for accurate assessment of the factors that contribute to insulin sensitivity and resistance in animal models of these conditions. Generally, insulin sensitivity of skeletal muscle, one of the major tissues for glucose disposal, is assessed from the relationship between plasma level of insulin and glucose uptake. However, plasma insulin concentration is not a good index of the concentration of insulin in the interstitial fluid (ISF) of skeletal muscle from where it accesses insulin receptors on the sarcolemma. Indeed, it is well established that the plasma insulin concentration required to increase glucose disposal rate *in vivo* is significantly higher than that required *in vitro* (Poulin, Steil, Moore, Ader, & Bergman, 1994). Moreover, the rate of increase in glucose uptake during hyperinsulinaemic-euglycaemic (HE) clamp is delayed compared to the change in plasma insulin concentration (Castillo, Bogardus, Bergman, Thuillez, & Lillioja, 1994).

The disparities between plasma and ISF insulin concentrations are

Abbreviations: ISF, interstitial fluid; HE, hyperinsulinaemic-euglycaemic clamp; TET, transendothelial transport; BSA, bovine serum albumin; I/P ratio, interstitial to plasma ratio; MI, insulin sensitivity; FEP, fluorinated ethylene propylene; ELISA, enzyme-linked immunosorbent assay; LBM, lean body mass; BW, body weight; MABP, mean arterial blood pressure; ABP, arterial blood pressure; BG, blood glucose; GIR, glucose infusion rate; HR, heart rate

* Corresponding author.
E-mail address: j.m.marshall@bham.ac.uk (J.M. Marshall).
[1] Cancer Sciences, University of Southampton, Southampton SO17 1BJ, United Kingdom.
[2] AstraZeneca, Cambridge Science Park, Cambridge CB4 0FZ, United Kingdom.
[3] Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool L3 3AF, United Kingdom.
[4] CrystecPharma Ltd., University of Bradford, BD7 1DP, United Kingdom.

http://dx.doi.org/10.1016/j.vascn.2017.04.001
Received 11 December 2016; Received in revised form 2 March 2017; Accepted 5 April 2017
Available online 06 April 2017

partly explained by the fact that insulin must cross the capillaries in order to reach the ISF. Trans-endothelial transport (TET) of insulin is regulated by an insulin-receptor mediated process on the endothelial cells. Insulin also acts on insulin receptors on resistance vessels and terminal arterioles to increase muscle blood flow and the capillary surface area for exchange (Barrett et al., 2009; Barrett, Wang, Upchurch, & Liu, 2011). Thus, the relationship between plasma ISF and ISF insulin is dependent on the sensitivity of the insulin receptors at these different vascular sites and these factors in turn, affect estimates of muscle insulin sensitivity. Nevertheless, a full appreciation of the sensitivity of skeletal muscle to insulin ultimately requires that insulin concentrations be measured in ISF.

Insulin concentration in lymph has been used as a surrogate for ISF insulin concentration in hindlimb of humans (Castillo et al., 1994) and rats (Poulin et al., 1994). Such measurements indicated lymph concentrations are substantially lower than those in plasma and correlate better with glucose uptake than plasma insulin concentration (Yang, Hope, Ader, & Bergman, 1989). However, lymph insulin concentration is not an accurate assessment of ISF insulin because lymph drainage from hindlimb derives from skin, adipose tissue and bone, as well as muscle. The alternative is to use microdialysis to directly assay insulin in muscle ISF as has been reported in humans and rats (*e.g.* Sjostrand, Holmang, Strindberg, & Lonnroth, 2000; Gudbjornsdottir, Sjostrand, Strindberg, & Lonnroth, 2005; Holmang, Mimura, Bjorntorp-, & Lonnroth, 1997). However, this is technically challenging particularly in rodents, because insulin is a large molecule that diffuses slowly across the pores of dialysis membranes, and because insulin concentration is notoriously difficult to measure in low concentrations in small fluid volumes. Indeed, the low concentrations of insulin in ISF under basal conditions have not yet been measured accurately in the rat.

Thus, the primary aims of the present study were to optimise the microdialysis methodology *in vitro* whilst employing validated techniques for assaying low concentrations of insulin in small fluid volumes. We then adapted these techniques so that they could be used accurately and reliably to measure ISF insulin concentrations *in vivo* in the rat. We also established than an inulin infusion given from 80 min before the start of the experiment proper, could serve as an effective reference for insulin. Our final aim was to measure ISF insulin concentration in hindlimb muscle of lean and obese Zucker rats in the basal state and during HE clamp so as to clarify the relationship between plasma and ISF insulin and to allow direct estimates of skeletal muscle insulin sensitivity.

2. Methods

2.1. Validation of the microdialysis method in vitro

Factorial experimental design experiments were performed to determine the best conditions for recovery of insulin. All equipment was purchased from CMA Microdialysis, Sweden *via* Linton Instrumentation, Norfolk, UK unless stated otherwise.

2.1.1. Reagent preparation

All reagents were prepared on the day of study unless stated otherwise.

Microdialysis perfusion fluid was prepared using 10% BSA and 20% (w/v) glucose. A working solution of 1% BSA and 1.5 mmol/l glucose was prepared in 0.9% sodium chloride and delivered at 1 μl/min.

Inulin A stock solution of 10 mg/ml inulin was diluted in 0.9% sodium chloride to a solution concentration of 25 μg/ml.

Human insulin was prepared by diluting human insulin (Actrapid®, Novo Nordisk, Denmark) in 2% BSA (in 0.9% sodium chloride) to concentrations of 7000, 3000, 1000 and 300 pmol/l.

2.1.2. Microdialysis probe preparation

Microdialysis probes (CMA 20), 10 mm in length with cut-off pore sizes of 55 or 100 kDa, were connected *via* fluorinated ethylene propylene (FEP) tubing and tubing adapters. The inlet FEP tubing was connected to a 1 ml microsyringe in an infusion pump (CMA 402 dual syringe pump) filled with perfusion fluid (see above). Insulin was diluted to 7000 pmol/l as detailed above and 1 ml of solution was placed in siliconised glass vials. Glass vials were secured in a CMA 130 *in vitro* stand and microdialysis probes were submerged in test solution and secured with the clips provided. Probes were equilibrated for 40 min with each new set of conditions. The rate of perfusion was 1, 2 or 2.5 μl/min. All equipment and tubing was either treated with Sigmacote® (Sigma-Aldrich Ltd., UK), or it was not. Following initial experiments to test the optimum conditions for insulin recovery, further experiments were conducted to assess recovery at different concentrations of insulin (3000, 1000 and 300 pmol/l) and inulin (25 μg/ml).

2.1.3. Sample collection and analyses

The perfusate was collected in either plastic, or glass vials: 40 min samples were collected, split equally for storage and analysed in duplicate. Insulin and inulin were assayed with ELISA Kits (see Section 2.2.7).

2.2. Refinement of microdialysis methodology in vivo

All experiments were conducted in accordance to the Animal Scientific Procedures Act 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. They were performed on either male Wistar rats, or male lean or obese Zucker rats (supplied by Charles River, Kent, UK). Anaesthesia was induced with an intraperitoneal (i.p.) injection of 160–190 mg/kg sodium-thiobutabarbitol (Inactin®, Sigma-Aldrich Ltd., UK) and supplemented as required, with bolus doses of 0.5 ml, or by increasing the infusion rate of anaesthetic *via* a cannula placed in the jugular vein. At the end of the experimental protocol, the animal was humanely killed by overdose of sodium-thiobutabarbitol. Death was confirmed by cervical dislocation.

2.2.1. Experimental groups

2.2.1.1. Inulin infusion. Experiments were performed on 3 anaesthetised male Wistar rats (weight 222 ± 15 g) to establish whether inulin could be given by infusion so as to provide a reference for insulin. The inulin infusion protocol was based on modelling of kinetics in preliminary experiments not reported here. Thus, inulin was given as a 50 mg/kg bolus followed by infusion at 2 mg/kg/min for 180 min. Blood samples for analysis were taken at 1 and 5 min and then at 15 min intervals. Each sample was centrifuged at 13, 000 RPM for 5 min at 4 °C and plasma was stored at 5 °C and analysed < 1 week after collection. In the preliminary inulin kinetic experiments, we tested whether samples could be frozen prior to analysis of inulin; these findings are shown in Supplementary data Fig. 1.

2.2.1.2. Combined inulin infusion and HE clamp. Experiments were performed on 4 male Wistar rats (weight 222 ± 15 g); see Supplementary data for details. The methodology tested in these experiments was adopted for the experiments proper and is described in detail below (see Sections 2.2.2–2.2.9). These experiments were also used to compare recovery of insulin with microdialysis probes at different pore cut-off sizes of 55 and 100 kDa. Therefore, half of the probes prepared and inserted as described in Sections 2.2.4 and 2.2.5 were 55 kDa and half were 100 kDa.

2.2.1.3. Use of validated microdialysis methodology during HE clamp. Experiments were performed on 6 lean and 5 obese Zucker rats. Full methodology is described below (see Sections 2.2.2–2.2.9).

2.2.2. Animal husbandry

Animals were housed in cages on a controlled 12 h Light/Dark