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Multiple Injections of Coloured Microspheres for Islet Blood Flow Measurements in Anaesthetised Rats: Influence of Microsphere Size[1]

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ABSTRACT

We investigated if coloured microspheres could be used for repeated measurements of pancreatic islet blood flow in rats. An initial injection of 1.0–1.5 × 10^5 microspheres (black colour), with a size of 10 or 15 μm, was made into the ascending aorta, while an arterial reference sample was collected from the femoral artery. Twelve min later, 1 ml of saline or 30% D-glucose was injected intravenously. Three min after this injection a second injection of 10- or 15-μm microspheres (green colour) was given. The animals were then killed, and the pancreas and adrenals were removed and samples (150–200 mg) were secured from the duodenum, ileum, colon, right kidney and liver. The microsphere contents were determined with the aid of a freeze-thawing technique and blood flow values were calculated. Our results suggest that 10-μm microspheres, but not 15-μm microspheres, provide reproducible islet and total pancreatic blood flow measurements when repeatedly injected. Values for the blood flow to the intestines, kidney and liver were less sensitive to the size of the microspheres. We conclude that repeated administration of 15-μm microspheres induces a high risk for erroneous islet and total pancreatic blood flow measurements, whereas two such measurements can be performed if 10-μm microspheres are used.

INTRODUCTION

Since the technique was introduced in 1967 by Rudolph and Heymann (1), polystyrene microspheres have been widely used to determine cardiac output and organ blood flow distribution in experimental animals. Several prerequisites have to be met to obtain reliable blood flow values, including that the microspheres must become adequately mixed with the arterial circulation, but not cause haemodynamic disturbances. Moreover, the microspheres must have rheological properties similar

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to those of erythrocytes but not shunt through the peripheral vascular bed. The number of microspheres trapped in the investigated organs must also be large enough to minimise the importance of the statistical variation compared to the biological variation. In our initial studies on pancreatic islet blood flow in rats (2, 3) and mice (4) we evaluated whether a single injection of microspheres could be used for quantitative studies. Since then, we and others (reviewed in 5) have repeatedly used this technique for studies of islet blood flow regulation. However, using this procedure the blood flow can only be determined once in each experiment. The aim of the present study was therefore to evaluate if repeated injection of 10- or 15 μm microspheres affects systemic and regional blood flow and if it is possible to use this technique for multiple islet blood flow measurements in rats.

MATERIALS AND METHODS

Animals: Adult, male Sprague-Dawley rats from a local breeding colony (Biomedical Centre, Uppsala, Sweden) with free access to tap water and pelleted food were used in all experiments. All experiments were approved by the local animal ethical committee.

Blood flow measurements: Measurements of blood flow were performed with a microsphere technique as previously described in detail (2). The rats were anaesthetised with an intraperitoneal injection of thiobutabarbital (Inactin^{TM}; Research Biochemicals, Natick, MA, USA; 120 mg/kg body weight), heparinised (200 IU heparin; Lövens Läkemedel, Malmö, Sweden) and placed on a heated operating table to maintain body temperature. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and vein. The intravenous catheter was used to continuously infuse Ringer solution (5 ml/kg × h). Constant monitoring of the mean arterial blood pressure was achieved by connecting the catheter in the ascending aorta to a pressure transducer (PDCR 75/1; Druck Ltd., Groby, UK). After stable blood pressure had been established (less than 10% variation during 20 min) 100.000–150.000 non-radioactive microspheres (E-Z Trac Ultraspheres^{R}; IMT, Stason Labs., Irvine, CA, USA), with a mean diameter of 10 or 15 μm , were suspended in 0.2 ml saline containing 0.001% (v/v) Tween 80, sonicated and injected through the cranial catheter during 10 sec. The colour of these microspheres was black. An arterial blood sample was collected by free flow from the catheter in the femoral artery, at a rate of approximately 0.40–0.50 ml/min, for a total of 60 sec starting immediately before the injection of microspheres. The exact flow rate was confirmed in each experiment by recording the weight of the sample. After obtaining the reference sample, an additional blood sample (~10 μl) was obtained from the femoral, intra-arterial catheter for measurement of blood glucose concentrations (see below). Twelve minutes after this first microsphere injection an intravenous injection of 1 ml saline or 30% (wt/vol) D-glucose was given. After 3 min further, *i.e.* 15 min after the first microsphere injection, a second injection of 150.000–200.000 microspheres with green colour (IMT)

was administered as described above. The size of the microspheres in this second injection was the same as that given at the first, *viz*. 10 or 15 μm. Thereafter the animals were killed by an intravenous injection of saturated KCl. The pancreas and adrenal glands were removed *in toto*, blotted, weighed and then subjected to a freeze-thawing technique to visualise the microspheres (6). Furthermore, samples from the duodenum, terminal ileum, descending colon, anterior liver lobe and a portion from the mid-region of the right kidney, a slice encompassing both cortex and medulla (approximately 150–200 mg each), were removed and treated by the same freeze-thawing technique. The number of microspheres present in the organs was counted in a microscope equipped with both bright and dark field illumination (6–8). Moreover, the percentage of pancreatic islets containing black and green microspheres was determined. The microsphere content in the arterial blood reference samples was directly counted in a light microscope after transferring the blood to glass microfibre filters (pore size <0.2 μm). In all these estimations of microsphere contents the black and green coloured microspheres could easily be distinguished, and thereby counted separately. The blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$ where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of reference sample (ml/min), Norg is number of microspheres present in the organ and N_{ref} is number of microspheres in the reference sample. A difference <10% in blood flow values between the adrenal glands was regarded to indicate an adequate dispersal of microspheres in the circulation.

Measurements of blood glucose and serum insulin concenterations: Arterial blood samples were obtained after securing the reference blood sample and later analysed for blood glucose concentrations with a blood glucose meter (Medisense®; Baxter Travenol Laboratories Inc., Deerfield, IL, USA) and serum insulin concentrations with ELISA (Rat Insulin ELISA®; Mercodia AB, Uppsala, Sweden) with rat insulin (Novo Nordic, Bagsværd, Denmark) as a standard.

Statistical calculations: All values are expressed as means ± SEM. Calculations of statistical significance were performed with Student's t-test for unpaired observations or single factor factorial ANOVA in conjunction with Bonferroni's test by use of Sigmastat® (SSPD; Erfart, Frankfurt, Germany). $P<0.05$ was considered to be statistically significant.

RESULTS

Injection of 10 μm microspheres followed by saline administration did not influence either mean arterial blood pressure (Table 1) or blood flow to the duodenum, ileum, colon, adrenals, kidney or the hepatic arterial bed when compared to measurements by a second 10 μm-microsphere injection in the same animals (Table 2). Neither did this procedure affect the blood flow to the whole pancreas (Figure 1) or the pancreatic islets (Figure 2). Injection of 15 μm microspheres followed by saline administration slightly decreased mean arterial pressure (Table 3), but did not affect the blood perfusion of the duodenum, ileum, colon, adrenals, kidney or

Table 1. Hematocrit, mean arterial blood pressure, blood glucose and serum insulin concentrations in association with blood flow measurements with 2 microsphere injections (10 µm-spheres; 100,000–200,000 each) separated by 15 min. The first injection was made with black, the second with green microspheres. Three min before the second injection 1 ml saline or 30% glucose was given intravenously.

Pretreatment	Saline		Glucose	
Injection	First	Second	First	Second
No of animals	8	8	8	8
Hematocrit (%)	ND	44.9 ± 0.5	ND	42.1 ± 0.5§
Mean arterial blood pressure (mm Hg)	107 ± 4	106 ± 4	107 ± 4	107 ± 4
Blood glucose (mmol/l)	ND	6.0 ± 0.2	ND	20.7 ± 0.5§§§
Serum insulin (ng/ml)	ND	3.45 ± 0.43	ND	8.96 ± 1.02**

Values are means ± SEM. ** denotes P<0.01 when compared to the corresponding saline-treated rats. § denotes P<0.05 and §§§ P<0.001 when compared to the corresponding saline-pretreated animal. ND is not determined.

hepatic arterial bed as measured by a second 15 µm-microsphere injection (Table 4). The blood flow to the whole pancreas (Figure 1) and preferentially the pancreatic islets (Figure 2) was, however, increased by this procedure as measured by a second 15 µm-microsphere injection. Administration of glucose after the first

Table 2. Organ blood flow values in association with blood flow measurements with 2 microsphere injections (10 mm-spheres; 100,000–200,000 each) separated by 15 min. The first injection was made with black, the second with green microspheres. Three min before the second injection 1 ml saline or 30% glucose was given intravenously.

Pretreatment	Saline		Glucose	
Injection	First	Second	First	Second
No of animals	8	8	8	8
Duodenal blood flow (ml/min × g)	2.29 ± 0.38	2.18 ± 0.30	2.37 ± 0.30	2.98 ± 0.29
Ileal blood flow (ml/min × g)	1.15 ± 0.25	1.27 ± 0.22	0.95 ± 0.19	1.52 ± 0.32*
Colonic blood flow (ml/min × g)	1.83 ± 0.29	1.54 ± 0.17	0.65 ± 0.19	1.02 ± 0.16*
Adrenal blood flow (ml/min × g)	3.26 ± 0.29	3.56 ± 0.48	3.06 ± 0.38	3.70 ± 0.34
Renal blood flow (ml/min × g)	4.05 ± 0.69	4.30 ± 0.65	3.64 ± 0.77	4.44 ± 0.50
Arterial liver blood flow (ml/min × g)	0.19 ± 0.06	0.21± 0.06	0.16 ± 0.04	0.25 ± 0.03*

Values are means ± SEM. * denotes P<0.05 when compared with the first injection of animals receiving the same-pretreatment.

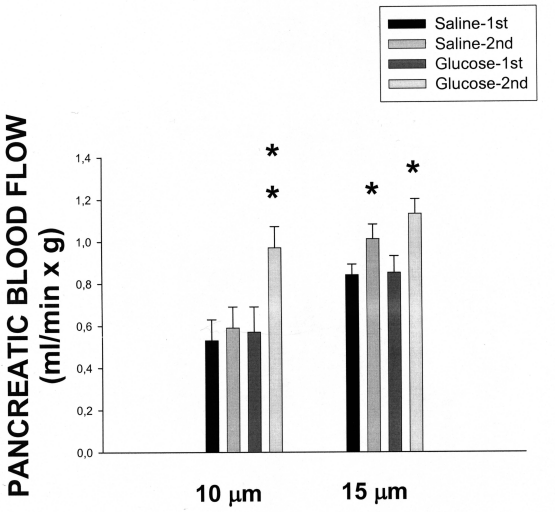

Figure 1. Whole pancreatic blood flow in anaesthetised adult male Sprague-Dawley rats. Blood flow measurements were performed by two injections of either 10- or 15-μm-microspheres before (Saline-1st, Glucose-1st) or after intravenous injection of 1 ml of saline (Saline-2nd) or glucose (Glucose-2nd). * denotes P<0.05 and ** P<0.01 when compared to corresponding animals before administration of saline or glucose.

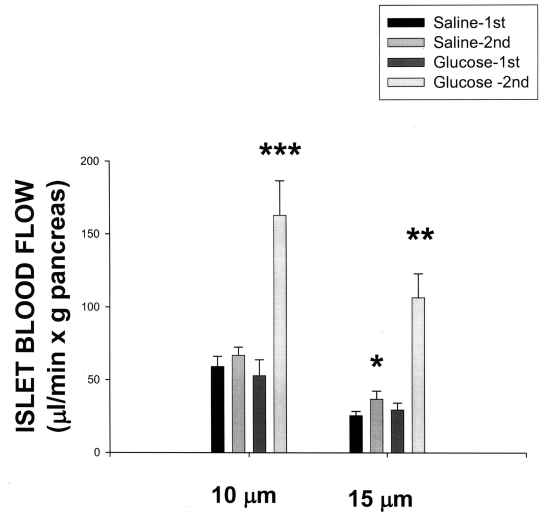

Figure 2. Islet blood flow in anaesthetised adult male Sprague-Dawley rats. Blood flow measurements were performed by two injections of either 10- or 15-μm-microspheres before (Saline-1st, Glucose-1st) or after intravenous injection of 1 ml of saline (Saline-2nd) or glucose (Glucose-2nd). * denotes P<0.05, ** P<0.01 and *** P<0.001 when compared to corresponding animals before administration of saline or glucose.

Table 3. Hematocrit, mean arterial blood pressure, reference sample velocity, blood glucose and serum insulin concentrations in association with blood flow measurements with 2 microsphere injections (15 μm-spheres; 100,000–200,000 each) separated by 15 min. The first injection was made with black, the second with green microspheres. Three min before the second injection 1 ml saline or 30% glucose was given intravenously.

Pretreatment	Saline		Glucose	
Injection	First	Second	First	Second
No of animals	9	9	7	7
Hematocrit (%)	ND	46.8 ± 0.8	ND	42.0 ± 1.1§§
Mean arterial blood pressure (mm Hg)	140 ± 3	129 ± 4**	136 ± 4	134 ± 6
Blood glucose (mmol/l)	ND	6.3 ± 0.2	ND	22.6 ± 0.3§§§
Serum insulin (ng/ml)	ND	3.98 ± 0.56	ND	9.98 ± 1.24§§§

Values are means ± SEM. ** denote P<0.02 and *** P<0.001 when compared with the first injection of animals receiving the same-pretreatment. §§ denote P<0.01 and §§§ P<0.001 when compared to the corresponding saline-pretreated animal. ND is not determined.

microsphere (10 or 15 μm) injection increased both blood glucose and serum insulin concentrations when measured immediately after the second microsphere injection, but did not influence mean arterial blood pressure (10 μm; Table 1 and 15 μm; Table 3). Ileal, colonic and arterial liver blood flows were increased by glucose, whereas duodenal, adrenal and renal blood flows were unaffected, as mea-

Table 4. Organ blood flow values in association with blood flow measurements with 2 microsphere injections (15 μm-spheres; 100,000–200,000 each) separated by 15 min. The first injection: was made with black, the second with green microspheres. Three min before the second injection 1 ml saline or 30% glucose was given intravenously.

Pretreatment	Saline		Glucose	
Injection	First	Second	First	Second
No of animals	9	9	7	7
Duodenal blood flow (ml/min × g)	2.09 ± 0.24	2.00 ± 0.18	2.21 ± 0.30	2.90 ± 0.20
Ileal blood flow (ml/min × g)	1.42 ± 0.16	1.47 ± 0.12	1.23 ± 0.23	1.22 ± 0.13
Colonic blood flow (ml/min × g)	1.83 ± 0.29	1.54 ± 0.17	1.60 ± 0.23	2.37 ± 0.45
Adrenal blood flow (ml/min × g)	5.93 ± 0.27	4.94 ± 0.88	6.42 ± 1.53	7.61 ± 0.01
Renal blood flow (ml/min × g)	4.97 ± 0.59	3.94 ± 0.25	3.97 ± 0.38	3.77 ± 0.30
Arterial liver blood flow (ml/min × g)	0.32 ± 0.10	0.35± 0.09	0.38 ± 0.14	0.34 ± 0.11

Values are means ± SEM.

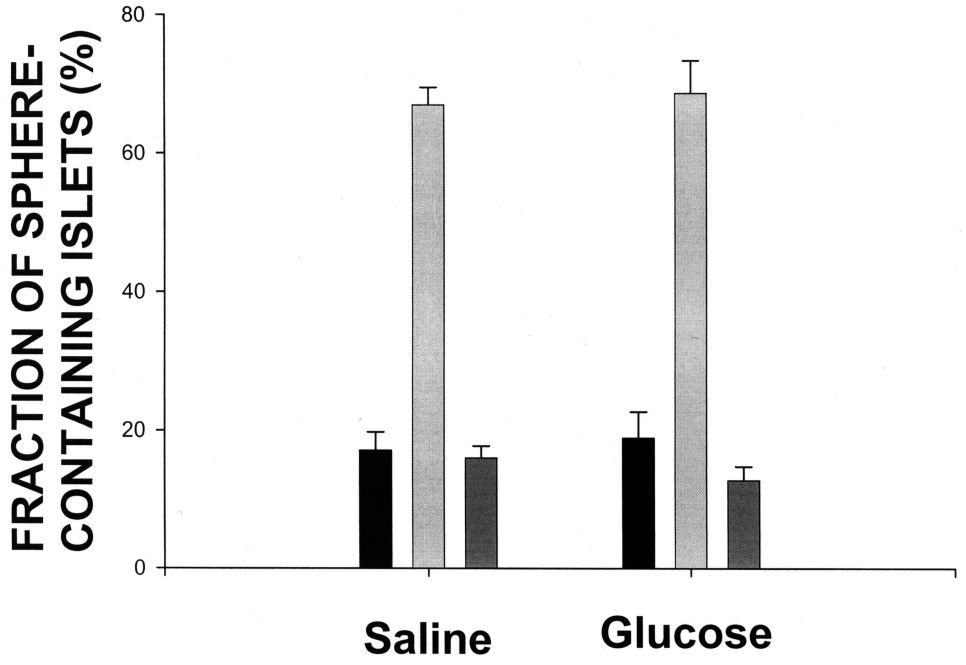

Figure 3. Fraction of pancreatic islets of adult male Sprague-Dawley rats containing 10 µm microspheres from first (black bar), first or second (light grey bar) or both (dark grey bar) microsphere injections.

sured by a second 10 µm-microsphere injection (Table 2). In contrast, none of these blood flow values were affected by glucose as measured by a second 15 µm-microsphere injection (Table 4). Whole pancreatic blood flow (Figure 1) and preferentially islet blood flow (Figure 2) was increased by glucose administration as measured both by repeated 10- and 15 µm-microsphere injections. Islets containing one or more microspheres after the first injection in almost all cases also contained microspheres from the second injection in both saline- and glucose administered animals (Figure 3)

DISCUSSION

The introduction of commercially available coloured microspheres (9,10) made repeated measurements of regional blood perfusion in experimental animals possible. This provides several advantages, e.g. paired analysis of measurements and a reduction of the number of animals needed. In a recent study, repeated injections of differently coloured 10 µm-microspheres were found to give reproducible islet blood flow measurements in awake rats (11). The present study evaluated this finding in anaesthetised rats and whether such experiments are restricted to the use of 10 µm microspheres or also applicable for 15 µm microspheres. Moreover, we also

investigated whether the distribution of blood flow between different islets was affected by this procedure.

Rheologically, microspheres should behave as erythrocytes to give reliable blood flow values. Over the size range of 10–15 μm the bias in size of microspheres compared to erythrocytes appears unimportant for most organs (12,13). To avoid the risk of arteriovenous shunting 15 μm microspheres have become the standard choice. For islet blood flow measurements, however, 10 μm microspheres have proven to provide higher and more reliable blood flow values (3, 14). This is likely to be due to the more centripetal distribution in blood vessels of larger particles (15, 16), which thereby are less prone to enter the afferent islet vessels in quantities proportional to red blood cells. Consistent with this, the present results show an apparent redistribution of microspheres from the islets to the exocrine parenchyma after measurements with 15 μm microspheres. A second injection of 15μm microspheres, but not 10 μm microspheres, after saline administration caused a decreased mean arterial blood pressure and an increase in both whole pancreatic and islet blood flow. Other recorded blood flows were, however, unaffected. This indicates that repeated 15 μm microsphere injections may be inappropriate for pancreatic and islet blood flow measurements due to arterial blockage by the first injection in some vascular beds and subsequent redistribution of blood into the pancreas. Individual islets with a high blood perfusion could similarly be obstructed by the first microsphere injection. However, when using the 10 μm microspheres, this did not seem to occur, since islets containing one or more microspheres after the first injection in almost all cases also contained microspheres from the second injection.

The blood glucose concentration seems to be the most important single factor for the control of islet blood perfusion (5). In the present work, there was an increased whole pancreatic blood flow, and preferentially islet blood flow, after glucose administration as measured by a second microsphere injection. Similar findings were obtained irrespective of whether 10 or 15 μm microspheres were used. However, ileal, colonic and arterial liver blood flows were increased after glucose administration only when measured by repeated injections of 10 μm microspheres. Glucose is well known to induce splanchnic hyperaemia after oral administration, but this effect is mainly locally mediated (17, 18). Glucose may also affect splanchnic blood perfusion when administered intravenously (19), although we in most studies have not recorded such effects. Normally, there are large variations in splanchnic blood flow values between different animals, a problem which may have been circumvented by using the present technique with repeated 10 μm microsphere injections to provide paired observations.

In conclusion, repeated administration of 15-μm microspheres induces a high risk for erroneous islet and total pancreatic blood flow measurements by the second injection, whereas two such blood flow measurements can be performed if 10-mm microspheres are used. This may be advantageous by providing paired observations of measurements and thereby reduce the number of animals investigated.

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