Upsala J Med Sci 83: 163–166, 1978

Microsphere Measurement of Regional Intestinal Blood Supply and Cardiac Output in the Rat

KARIN NORLÉN, LEIF RENTZHOG and STIG WIKSTRÖM

From the Departments of Anaesthesiology, Surgery and Clinical Research II, University Hospital, Uppsala

ABSTRACT

The regional blood supply to consecutive segments of the small intestine in the anaesthetized rat was investigated with a radioactive microsphere technique. A blood flow gradient with the lowest flow in the distal segments (0.85–0.89 ml/min·g) and the highest in the proximal segments (1.13–1.15 ml/min·g) was observed. Very few microspheres were found in the portal vein blood, indicating negligible arteriovenous shunting in the splanchnic area. The mean cardiac outputs in two consecutive measurements were 27.9 and 28.7 ml/min·100 g, respectively. The cardiac output and regional blood flow values were in accordance with those obtained with other techniques.

INTRODUCTION

The indicator fraction technique, with use of 86-rubidium and 42-potassium, and the microsphere technique have been employed in recent years to study the regional distribution of the blood flow and cardiac output in the rat and other small experimental animals. The regional blood flow in the rat intestine has been measured with the former technique (13, 15). This method has the disadvantage, however, that neither serial determinations nor simultaneous cardiac output measurements can be made in the same animal. It was desirable to have a model by which the changes in the regional blood supply and central circulatory variables could be followed in connection with later experimentally induced ischaemia in the small intestine. The microsphere technique seemed suitable for this purpose. The investigation was therefore undertaken to determine the cardiac output and elucidate the blood supply to different parts of the small bowel with radioactive microspheres and to compare the results with those obtained with other techniques.

MATERIAL AND METHODS

The experiments were carried out on male Sprague-Dawley rats (AB Anticimex, Stockholm, Sweden) weighing 184–265 g. The animals were given a standardized laboratory diet (Astra-Ewos, Södertälje, Sweden) and were kept under uniform conditions. They were acclimatized in the laboratory for at least one week and were starved for 18 h before the experiment. Water was allowed ad libitum.

The animals were anaesthetized with thiobutabarbital sodium (Inactin® Byk-Gulden, West Germany) given intraperitoneally in a dose of 120 mg/kg body weight. They were then tracheotomized and placed on a servocontrolled heating pad. The rectal temperature was kept at 36° to 38°C. A polyethylene catheter was inserted into each femoral artery. The blood pressure was monitored continuously from the right femoral artery using a Statham transducer (P 23 Dc) and a Grass recorder. Animals with an initial mean arterial blood pressure (MABP) below 95 or exceeding 135 mmHg were excluded. The catheter in the left femoral artery was connected to a suction pump (Sage Instruments Syringe Pump 352) and used for taking reference blood samples during the microsphere injections. A polyethylene catheter with an outer diameter of about 0.8 mm was inserted into the right carotid artery and advanced until its tip lay in the aortic root. This catheter was used for microsphere injections.

Carbonized microspheres labelled with 141-cerium and 85-strontium, respectively, and measuring 15 ± 5 μm (3M Co, St Paul, Minn., USA), delivered in saline, were used. Immediately before the injection they were suspended in fresh rat plasma to a total volume of 0.4 ml and agitated in Vortex JR Mixer. 200 000–400 000 microspheres were injected each time. 85-Sr and 141-Ce labelled microspheres were given alternatly as the first and second injection in order to avoid systematic errors due to any difference in the size of the spheres.

About one hour after induction of anaesthesia the first microspheres, suspended in plasma, were injected through the catheter in the carotid artery in 15 sec. A reference blood sample of the same quantity as the microsphere suspensions was drawn at a constant rate (0.64 ml/min), starting 15 sec before the microsphere injection and ending 15 sec after its completion. The blood pressure recording was carefully observed during the injection and animals whose blood pressure was considerably affected (>10 mm) were excluded from the series. A second microsphere injection was given after another 30 min, and a reference blood sample was drawn in the same way.

The rat was then killed and the heart, lungs, kidneys, liver, stomach and small intestine were removed. The

Table I. *General conditions and haemodynamics in 15 rats*

Values are given as mean±S.E.M.

	First injection	Second injection
Cardiac output, ml/min·100 g	27.9±1.9	28.7±2.1
Kidney, left, ml/min·g	4.35±0.32	4.25±0.29
Kidney, right, ml/min·g	4.87±0.45	4.57±0.46
Liver, ml/min·g	0.63±0.07	0.65±0.07
Stomach, ml/min·g	0.56±0.09	0.55±0.06
Small intestine, ml/min·g	1.01±0.09	1.07±0.10
Lung, % of inj.	1.3 ±0.2	1.0 ±0.1
Mean arterial blood pressure, mmHg	117 ±3	113 ±3
Temperature, °C	36.8±0.1	36.9±0.1
Body weight, g	230±6	

small intestine was divided into 10 segments, each segment corresponding to a defined number of mesenteric vascular end-arcades. The different tissue specimens were weighed and the reference blood samples were centrifuged. The specimens and samples were then analysed with respect to their contents of 85-Sr and 141-Ce in a gammaspectrophotometer (Nuclear Chicago 1087). Pilot studies had shown that with the sample volumes used in the present experiments, no corrections for geometric differences were necessary. The total amount of microspheres injected was determined from the difference between the activity in the syringe before and after the injection.

The blood flow in the different tissues (f) was calculated as:

$$f = m \cdot \frac{f_s}{m_s}$$

where m is the activity in the actual tissue, m_s the activity in the reference blood sample and f_s the blood sampling rate. Cardiac output (CO) or more precisely cardiac output minus the coronary blood flow, was calculated in the same way, but here the factor m was the amount of activity injected minus the activity in the heart.

In two rats the possibility of arteriovenous shunting of blood in the splanchnic area was examined. After the pre-experimental preparations, as described above, these rats were laparotomized and the portal vein carefully dissected. During the microsphere injection into the carotid artery blood was drawn through a needle placed in the portal vein. This blood was analysed together with the other samples in the gammaspectrophotometer.

In four rats the acid base status was determined immediately after the first microsphere injection and 30 min, 60 min and in one case 120 min later. An automatic microanalyser (BMS 3 MK 2 Blood microsystem, Radiometer, Copenhagen, Denmark) was used for determination of pH and $PaCO_2$. Standard bicarbonate and base excess were then calculated.

In the statistical analysis of the results the mean, standard deviation (S.D.) and standard error of the mean (S.E.M) were calculated. Comparisons were made by means of Student's *t*-test for paired observations.

RESULTS

The haemodynamic characteristics 1 h and 1½ h, respectively, after induction of barbiturate anaesthesia are shown in Table I and Fig. 1. Weights are given as g wet tissue.

Cardiac output was 27.9±7.2 ml/min·100 g (mean±S.D.) at the first injection and 28.7±8.0 ml/min·100 g at the second. There was no significant difference between the two measurements. The mean arterial blood pressure during the first injection was 117±12 mmHg (mean±S.D.) and during the second injection 113±12 mmHg. The blood flows to the left kidney were 4.35 ml/g and 4.25 ml/g at the first and second injections, respectively, and to the right kidney 4.87 ml/g and 4.57 ml/g. There were no differences in the blood flows to the right and left kidneys during the same injection nor were there any differences in the flows to the respective kidneys between the two injections. The blood flows in the small intestine were calculated to be 1.01 ml/min·g and 1.07 ml/min·g, respectively. The proximal third of the intestine had higher blood flows, 1.15 ml/min·g and 1.13 ml/min·g than the distal third where the flow was 0.89 ml/min·g at the first injection and 0.85 ml/min·g at the second.

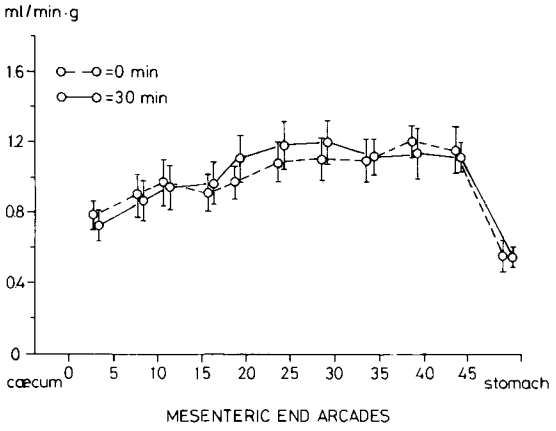

Fig. 1. Regional blood flows (ml/min·g) in the different segments of the small intestine and in the stomach. The numbers of mesenteric vascular end-arcades, counted from the caecum, are given on the abcissa.

Table II. *Acid-base status during barbiturate anaesthesia*

0 min refers to the measurement time immediately after the first microsphere injection (mean±S.E.M)

n	Time (min)	pH	$PaCO_2$ (kPa)	HCO_3 (mmol/l)	Base excess (mmol/litre)
4	0	7.47±0.01	4.8±0.3	26.3±1.3	+2.1±1.5
4	30	7.43±0.02	4.6±0.2	24.1±0.7	−0.3±0.9
3	60	7.42±0.02	5.1±0.2	24.1±0.7	+0.1±1.0
1	120	7.43	4.7	23.6	−0.8

The blood flows to the stomach, 0.56 and 0.55 ml/min · g, were lower than those to the intestine. There was no increase in the blood flow to the liver during the experimental period. The blood drawn from the portal vein contained a negligible amount of microspheres. The activities in the lungs at the first and second injections were 1.3% and 1.0% of the injected activity.

The results of the acid-base determinations are presented in Table II. No fundamental changes were noted during the experimental period.

DISCUSSION

For the microsphere method to be reliable, the microspheres must be completely mixed with the blood and distributed in proportion to the blood flow to the different tissues, where they are trapped by the capillary beds and do not recirculate (16). The tip of the catheter through which the microspheres were injected in the present experiments was placed in the aortic root. With this catheter position, in dogs, Kaihara et al. (4) have demonstrated complete mixing of the microspheres and blood, except for the fraction to the heart. In our experiments this position also appeared to give adequate mixing in the rat. Thus, there was an equal distribution to the kidneys during the same injection and all organs other than the heart showed the same distribution of the two radionucleides. However, as the heart values were unreliable, the cardiac output was calculated after subtraction of the activity in the heart and therefore in fact comprises cardiac output minus coronary blood flow. Our cardiac output values are thus slightly underestimated, but they compare favourably with other results obtained with different techniques (7, 14, 3, 6). The coronary blood flow in the rat has been estimated at 2.9% (11) and 6.4% (12) of the cardiac output.

Few microspheres seemed to escape trapping and pass through arteriovenous shunts as judged by the radioactivity in the lungs, which at the two injections was 1.3% and 1.0% of the total amount injected and this probably mainly reflects the bronchial circulation. In agreement with this result Mendell & Hollenberg (8), who injected 35 μm spheres into the aorta at the level of the diaphragm, observed a delivery to the lungs of only 0.04% of the cardiac output. In a report on experiments in the dog, Kaihara et al. (4) pointed out that barbiturate anaesthesia probably results in opening of arteriovenous shunts with considerable trapping of 15 μm microspheres in the lungs. This effect was not observed in the rat in the present investigation.

The microsphere technique has been employed to determine the regional blood flow in the gastrointestinal tract, mostly in larger animals. Grim & Lindseth (2) used glass microspheres to calculate the blood flow in dog intestine. Greenway & Murthy (1) investigated with microspheres the distribution of the superior mesenteric arterial flow in the cat. They found a uniform flow of 0.85 ml/min · g along the length of the small intestine.

The total blood flow in the small intestine calculated in the present study is almost the same as that reported by Sasaki & Wagner (12) from their investigation in the rat. In accordance with Steiner & Mueller (13) and Varga & Csáky (15), both of whom used the 86-Rb indicator fractionation technique, we found a higher blood flow in the proximal than in the distal part of the intestine. Varga & Csáky (15) pointed out that the blood supply gradient develops progressively with age, and in rats aged 6 months or older the blood flow is significantly lower in the ileum than in the jejunum. The blood flows to the intestinal segments in the present study compare well with the results of these latter authors obtained with the 86-Rb technique. One possible source of

error in using the microsphere technique to determine regional intestinal blood flow, however, may be a heterogeneous distribution of the spheres caused by plasma skimming. This probably has significance only for the distribution of the spheres between the different parallel vascular systems within the intestinal wall, and will not influence the total flow in an intestinal segment.

The arterial blood supply to the liver amounted to 0.63–0.65 ml/min · g, corresponding to 7% of cardiac output, which is in agreement with the findings of Lindell & Aronsen (5) and Norlén et al. (9). Mendell & Hollenberg (8) and Rakušan & Blahitka (10) reported a smaller fractional flow to the liver, i.e. 2–3% of the cardiac output. With the 86-Rb technique Sapirstein et al. (11) calculated the distribution to the liver to be 6.6%, but this probably includes the blood flow in both the portal vein and hepatic artery. By measuring the activity in the blood from the portal vein it could be excluded in our experiments, however, that the arterial blood flow to the liver was overestimated owing to arteriovenous shunting in the splanchnic area. The use of different anaesthetic compounds may be one reason for the variations in reported arterial liver blood flow.

ACKNOWLEDGEMENT

This work was supported by the Swedish Medical Research Council (project no. B78-17X-02809-08).

REFERENCES

1. Greenway, C. V. & Murthy, V. S.: Effects of vasopressin and isoprenaline infusions on the distribution of blood flow in the intestine; criteria for the validity of microsphere studies. Br J Pharmacol *46:* 177, 1972.
2. Grim, E. & Lindseth, E. O.: Distribution of blood flow to the tissues of the small intestine of the dog. Univ Minn Med Bull *30*: 138, 1958.
3. Hellberg, M., Källskog, Ö. & Wolgast, M.: Effect of hemorrhage on single glomerular blood-flow as related to central hemodynamics and acid-base balance. Acta Anaesthiol Scand *17*: 27, 1973.
4. Kaihara, S., van Heerden, P., Migita, T. & Wagner, H. N., Jr: Measurement of distribution of cardiac output. J Appl Physiol *25*: 696, 1968.
5. Lindell, B. & Aronsen, K. F.: Changes in cardiac output distribution after liver dearterialization in the rat. Acta Chir Scand *143*: 207, 1977.
6. Malik, A. B., Kaplan, J. E. & Saba, T. M.: Reference sample method for cardiac output and regional blood flow determinations in the rat. J Appl Physiol *40*: 472, 1976.
7. Mandel, M. J. & Sapirstein, L. A.: Effect of angiotensin infusion on regional blood flow and regional vascular resistance in the rat. Circ Res *10*: 807, 1962.
8. Mendell, P. L. & Hollenberg, N. K.: Cardiac output distribution in the rat: comparison of rubidium and microsphere methods. Am J Physiol *221*: 1617, 1971.
9. Norlén, B. J., Engberg, A., Källskog, Ö. & Wolgast, M.: Intrarenal hemodynamics in the transplanted rat kidney. Kidney Int. In press.
10. Rakušan, K. & Blahitka, J.: Cardiac output distribution in rats measured by injection of radioactive microspheres via cardiac puncture. Can J Physiol Pharmacol *52*: 230, 1974.
11. Sapirstein, L. A., Sapirstein, E. H. & Bredemeyer, A.: Effect of hemorrhage on the cardiac output and its distribution in the rat. Circ Res *8*: 135, 1960.
12. Sasaki, Y. & Wagner, H. N., Jr: Measurement of the distribution of cardiac output in unanaesthetized rats. J Appl Physiol *30*: 879, 1971.
13. Steiner, S. H. & Mueller, G. C. E.: Distribution of blood flow in the digestive tract of the rat. Circ Res *9*: 99, 1961.
14. Takács, L. & Vajda, V.: Effect of serotonin on cardiac output and organ blood flow of rats. Am J Physiol *204*: 301, 1963.
15. Varga, F. & Csáky, T. Z.: Changes in the blood supply of the gastrointestinal tract in rats with age. Pfluegers Arch *364*: 129, 1976.
16. Wagner, H. N., Jr, Rhodes, B. A., Sasaki, Y. & Ryan, J. P.: Studies of the circulation with radioactive microspheres. Invest Radiol *4*: 374, 1969.

Received January 16, 1978

Address for reprints:

K. Norlén, M.D.
Department of Anaesthesiology
University Hospital
750 14 Uppsala
Sweden