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Extraction Methods for the Determination of Regional Renal Blood Flow

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ABSTRACT

To permit determinations of the single nephron filtration fraction at va-various levels of the cortex of the rat kidney, methods for single glomerular blood flow measurements were evaluated. Simultaneous measurements of the medullary blood flow were made with the different test substances. Intra-aortic slug injections of radioactive microspheres, 86-rubidium and 131-I-antipyrine were given in rats. The two kidneys were clamped 10 s after the injection, the pedicles were ligated and the kidneys were removed and deep-frozen. A reference arterial blood sample was drawn continuously during the 10-second period. In another series microspheres and 131-I-albumin were injected. The radioactivity of tissue samples and reference blood samples was analysed and the blood flow of cortical and medullary regions was calculated. The microspheres gave a blood flow of 6.65 ul/mg in outer cortex, 8.15 in mid-cortex and 4.91 ul/mg in inner cortex. Corresponding values with rubidium were 4.32 ul/mg, 4.56 ul/mg and 3.86 ul/mg and with antipyrine 2.14 ul/mg, 2.28 ul/mg and 1.97 ul/mg. In the outer stripe of the outer medulla a blood flow of 1.65 ul/mg was obtained with rubidium, 1.08 ul/mg with antipyrine and 0.75 ul/mg with labelled albumin. The corresponding values in the inner stripe of the outer medulla were 1.04 ul/mg, 0.66 ul/mg and 1.01 ul/mg and in the inner medulla 1.04 ul/mg, 0.66 ul/mg and 1.23 ul/mg. It is concluded that the microsphere technique gives the most reliable results for determination of the glomerular blood flow, but that both antipyrine and rubidium - although giving too low values - reflect the distribution pattern of blood flow within the cortical region. Rubidium seems to measure the blood flow reliably in all parts of the medulla and labelled albumin in the inner stripe and in the inner medulla.

INTRODUCTION

In experimental animal models the glomerular filtration rate seems to be highly dependent on the hydrostatic and oncotic pressures acting across the glomerular membrane (Källskog et al. 1975,Navar et al.1977). Under normal conditions the filtration process is only to a lesser degree blood-flow dependent, which means that the glomerular plasma flow and the glomerular filtration rate cannot be correlated with each other. Pre-glomerular and post-glomerular vascular resistance may change oppositely to one another without any alteration in the blood perfusion rate. However, the effective glomerular filtration pressure will change,resulting in an altered filtration rate. Studies have been performed in which the single glomerular filtration rate (SNGFR) and the single glomerular blood flow (SGBF) were measured simultaneously at various levels of the renal cortex (Ericson et al. 1979). Here a modified Hansen technique was used for determination of the SNGFR and this technique had to be combined with a method for determination of the SGBF. This paper describes the evaluation of methods in which microspheres,86-Rb and 131-I-labelled antipyrine were used as test substances. In the same experiments the plasma flow in the renal medulla was estimated.

METHODS

In a first series of experiments six male rats weighing 310 - 380 g were used. The animals were anesthetized with Inactin^{R} (120 mg/g body weight i.p.), tracheostomized and placed on a heated operation table. The right common carotid artery was catheterized,the tip of the catheter being placed just above the aortic valves in the aorta. The femoral vein and femoral artery were catheterized for infusions, recording of blood pressure and blood sampling. Non-activated clamps were placed around the renal artery and renal vein. Both ureters were catheterized for urine sampling. The catheter in the left femoral artery was connected to a syringe for reference sampling at a constant suction rate.

Radioactive microspheres (3M), rubidium and I-antipyrine were used simultaneously for the blood flow measurements. 0.3 million microspheres labelled with 85-Sr (15 microns) were suspended in 0.1 ml of rat plasma containing 86--Rb-chloride and 131-I-antipyrine.

Just after the reference sampling was started, the radioactive solution was given as a slug injection into the aorta and after exactly 10 s the two renal clamps were activated and the reference sampling was stopped. The renal pedicles were ligated and the kidneys were removed,weighed and deep-frozen.

The glomerular filtration of all kidneys was measured with ^{3}H-inulin

during four 15-min periods before the intra-aortic injection and the arterial pressure was checked before and during the injection.

From frozen sections of the kidney the cortex was cut into three parts. The medulla was also divided into three parts - the outer stripe and inner stripe of the outer medulla and the inner medulla.The tissue pieces were placed on small pre-weighed aluminium plates, and were dried in an oven at 150 ^{o}C for at least five hours and then weighed again. The dried tissue samples, the remaining kidney and the reference blood samples were analysed for their content of radioactivity in a gamma-spectrometer. The vials containing the samples were placed in a brass shield in the well-crystal of the gamma-spectrometer in order to avoid disturbances by the beta-emission from the rubidium radioisotope (Öbrink et al. 1959). The tissue blood flow was calculated from the formula: tissue blood flow (µl/(min·mg dry weight)= reference sampling rate (µl/min)· · radioactivity in tissue sample / radioactivity in reference sample. The results were then corrected to wet weights.

In a second series 13 rats also weighing 310-380 g were used. The experimental procedure was identical to that described above, but the injected solution now contained 141-Ce-labelled microspheres and 131-I-labelled albumin. In this series the cortical blood flow was not calculated by means of the radioactive albumin.

RESULTS

Series 1. The mean arterial blood pressure varied between 100 and 125 mm Hg. In some of the rats the slug injection caused a small drop in arterial pressure of some 5 mm Hg, which lasted for only 1-2 s. The glomerular filtration rate was 0.8908 $\pm$ 0.4589 ($\pm 1\sigma$) ml/min·g kidney.

Microspheres with a diameter of 15 µm are totally trapped within the tissue at one passage through the kidney (Källskog et al. 1971). The results of the calculations of total renal blood flow and cortical regional flows are given in Fig. 1 and Table 1. The corresponding blood flows calculated from the antipyrine and rubidium data are also given in Fig. 1 and Table 1. As these substances are also trapped in the medullary as well as the cortical regions the medullary blood flow could be calculated.

Series 2. The glomerular filtration rates and arterial blood pressures did not differ from those of series 1. In these experiments the accumulation of radioactive albumin in the medullary tissue was analysed and the blood flow in the different medullary regions was calculated. The results are given in Table 2 and Figure 2.

To make it possible to compare the distribution patterns of blood flow

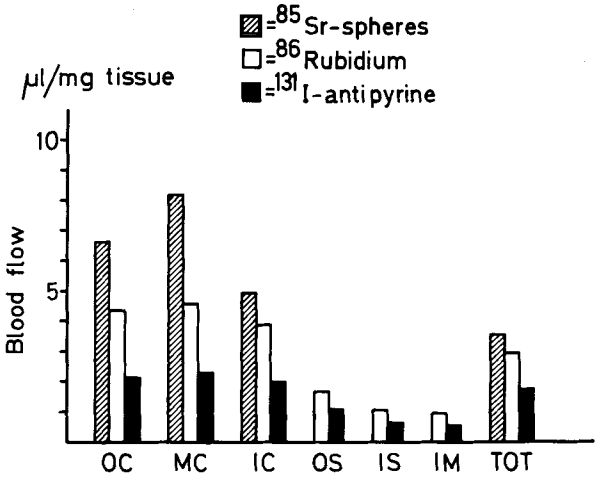

Fig. 1 The columns give the blood flow in cortical and medullary regions obtained with the use of 85-Sr-microspheres,86-rubidium and 131-I-antipyrine. OC = outer cortex,MC = mid-cortex, IC = inner cortex, OS = outer stripe of outer medulla,IS = inner stripe of outer medulla,IM = inner medulla.

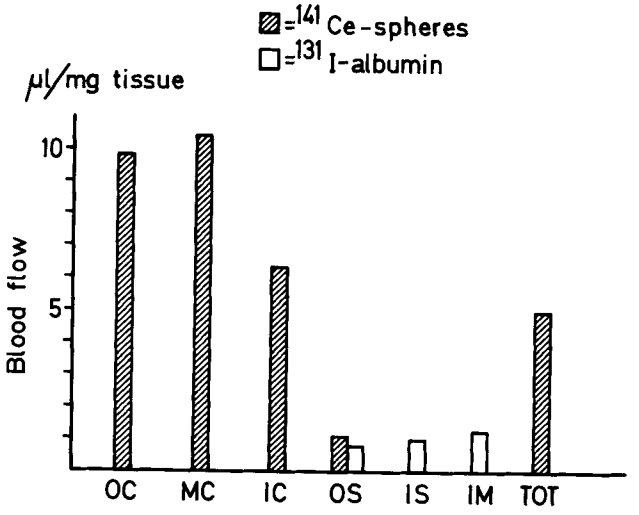

Fig. 2 The blood flow in cortical and medullary regions obtained with the microsphere and albumin accumulation techniques,respectively. For abbreviations,see Fig. 1.

TABLE 1

ul/mg tissue ± SEM

The total and regional renal blood flows obtained by means of 85-Sr-labelled microspheres,86-rubidium and 131-I-labelled antipyrine.

	OC	MC	IC	OS	IS	IM	TOT
85-Sr-spheres	6.65±0.898	8.15±1.009	4.91±0.709	0.952±0.092			3.52±0.377
86-Rubidium	4.32±0.539	4.56±0.495	3.86±0.406	1.65±0.174	1.04±0.111	0.944±0.096	2.93±0.303
131-I-antipyrine	2.14±0.256	2.28±0.244	1.97±0.202	1.08±0.112	0.655±0.054	0.545±0.048	1.76±0.170

TABLE 2

ul/mg tissue ±SEM

The total and regional renal blood flows obtained by means of 141-Ce-labelled microspheres and 131-I-albumin

	OC	MC	IC	OS	IS	IM	TOT
141-Ce-spheres	9.89±0.796	10.37±0.584	6.31±0.509	1.09±0.172			4.97±0.290
131-I-albumin				0.75±0.034	1.01±0.048	1.23±0.069	

calculated with the various test substances,all results have to be normalized. Normalized values were obtained by dividing the regional blood flow by the total blood flow measured with 85-Sr-microspheres,both given in µl/mig tissue,and are presented in Figure 3.

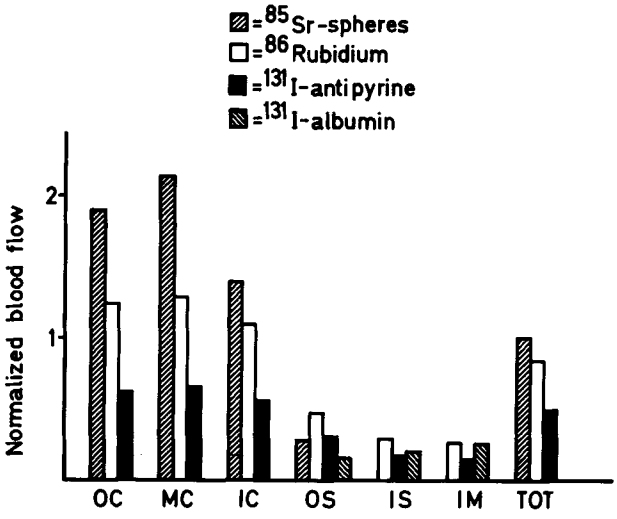

Fig.3 The columns give the normalized blood flow values in cortical and medullary regions,calculated as described in the text.For abbreviations,see Fig.1.

DISCUSSION

The pre-glomerular resistance is generally considered to be located in the afferent arterioles,but direct measurements of blood pressure within the interlobular arteries indicate a pressure drop along this artery,which means that the interlobular artery acts as a resistance vessel (Ulfendahl et al.,1975).This finding led to the assumption that the afferent arterioles belonging to deep glomeruli cause a large pressure drop of about 60 mm Hg,while the superficial afferent arterioles only cause a pressure drop of about 60 mm Hg,while the superficial afferent arterioles only cause a pressure drop of some 25 mm Hg. These differences in resistance properties of afferent arterioles might give quantitative and qualitative differences in the regulation of the blood flow and the glomerular filtration rate in deep and superficial regions.

The glomerular filtration rate of superficial and deep glomeruli can be measured with micropuncture techniques,but these measurements are restricted to certain animal species and to young animals.With the Hansen technique and its modifications the SNGFR can be determined in all cortical regions. A simplified modification of the Hansen technique has been used by the authors (Ericson et al. 1979) and has been combined with SGBF determinations by the microsphere

technique. In these studies it has been possible to calculate a single nephron filtration fraction at various levels. The choice of methods for measuring regional blood flow is a delicate problem which has been under debate in the literature for many years. Recently Aukland (1977) critically evaluated various methods.The microsphere technique has many potential virtues,but much criticism has been raised against it. As the microspheres of the size used in this investigation are trapped in the glomerular capillary tufts,the results reflect the glomerular blood flow at various levels of the cortex.The objection that the microsphere technique overestimates the blood flow in the outermost cortex cannot be fully neglected,but the low error of the method does not seem to invalidate its use. Thus Källskog et al.(1976) showed in the superficial glomeruli that SGBF calculated from SNGFR and the single nephron filtration fraction, obtained by micropuncture techniques was very similar to the values obtained with the microsphere technique.

The accumulation of radioactive potassium and rubidium (Sapirstein 1958, Harsing et al.1965) has been used as a means of measuring the regional blood flow in cortical and medullary regions. Our results with the rubidium method did not agree with those of the microsphere method.The rubidium values in the cortical regions were far below those obtained with microspheres,which means that the extraction during the 10-second period is much lower than 100 per cent. The cortical extraction of radioactive antipyrine is still less than for rubidium and the calculated blood flow values are,accordingly,small in comparison with the microsphere and rubidium values. Yarger et al. (1978) corrected for the rubidium extraction and considered that the method adequately estimated the regional blood flow. Their distribution pattern of blood flow does not agree with ours,but this may partly be explained by differences in the definition of the cortical layers. Moreover,they assume that the extraction of the rubidium is the same in all regions of the kidney,which might cause an error.

Apart from the absolute blood flow values,the distribution patterns with the three techniques do not differ to any major extent. Thus the midcortical zone shows the highest tissue flow,while the innermost zone gives the lowest low values. This might mean that the rubidium and the antipyrine methods,which measure the nutritive blood flow,also reflect the absolute blood flow within the three cortical regions. These two test substances cannot,however,be used in combination with SNGFR measurements to calculate the single nephron filtration fractions,whereas the SGBF values obtained are definitely too low.

As the microspheres are totally trapped in the glomeruli,they cannot be used for measurement of the medullary blood flow. To measure this flow we therefore used the accumulation of antipyrine,rubidium and albumin in the medulla.

It has been shown both in dogs (Lillienfield et al. 1961) and rats (Solez et al. 1974,Ganguli et al.1974), that a linear accumulation of albumin takes place in the inner medulla during a period of constant intravenous perfusion of the test substance. With the assumption that no labelled albumin leaves the inner medulla, the authors were able to calculate the plasma flow.Our modified method is based on the assumption that the entire bolus of the intra-aortic injection has reached the medullary regions and that no labelled albumin has left the region. In control experiments we opened the renal pelvis and the time of appearance of intravenously injected Evans blue solutions was measured in the papillary region. region. The appearance of the blue dye was observed visually.The appearance time amounted to 6-8 seconds.Under our conditions it therefore seems probable that the greater part of the injected test substances has reached the papillary tissue. It was assumed that the blood flowing into the medulla had the same hematocrit as systemic blood.

With the use of antipyrine much lower values were obtained both in the inner and outer medulla that with the use of albumin and rubidium. We believe that this is due to a counter-current exchange for the highly diffusible antipyrine in the vasa recta system resulting in decreased delivery of the antipyrine to the inner parts of the medulla. In the outer stripe of the outer medulla the value obtained with albumin was considerably lower than that with rubidium,while the values for the two test substances were the same in the inner stripe and in the inner medulla. This seems to us to be explained by the fact that the transit time for blood in the outer stripe is only a few seconds and that the intravascular labelled albumin to some extent has thus left the region during the test period of 10 seconds. The rubidium,however, is rapidly transported into the cells and is trapped there in a large potassium pool.

In the inner parts of the medulla the plasma transit time is much longer than in the outer parts and it seems probable that most labelled albumin entering the inner medulla is trapped there during the 10-second period. The similar results obtained with rubidium and labelled albumin in the two inner parts of the medulla give reason to believe in their reliability. The rubidium values agree well with the results of Carlberg et al.(1979),Soles et al.(1974) and Ganguli et al. (1974).

We conclude that the microsphere technique combined with the rubidium technique can give reliable information on the blood flow distribution to the cortex and to the medulla. We also consider the microsphere method of value in studies of blood flow distribution within the cortical structures,but believe that some distribution artefacts might exist.Rubidium is preferable for measuring the medullary plasma flows,but in the inner zone of the outer medulla and in the inner medulla the accumulation of labelled albumin will give the same values.

Radioactively labelled albumin is preferable under certain conditions,whereas 86-Rb is a beta-emitter,which can complicate the radioactive analysis.

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