Morphometry of the Glomerular Tuft During Normal Postnatal Growth in Female Rats. Effects of Age, Location of Glomeruli and Methods of Obtaining and Processing the Renal Tissue

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Summary

Total glomerular filtration rate depends upon the structural integrity, number and area of glomeruli. Counting the number of glomeruli as well as measuring glomerular area or volume is nowadays widely employed in human and experimental biology.

Although functional and morphological differences between superficial and juxtamedullary glomeruli have been described, this is not always taken into account in morphometric studies. The aim of this paper was to study in female rats the area of juxtamedullary glomeruli and the area of superficial glomeruli as well as the influence of the age of rats and the methods of obtaining and processing the renal tissue.

The glomerular area was larger in kidneys collected in liquid nitrogen and smaller in those fixed in Bouin (See Materials and Methods); 4% formaldehyde. These results were obtained in superficial and in juxtamedullary glomeruli ($F=29.60$, $p<0.0001$).

Glomerular area increased with time in superficial as well as in juxtamedullary glomeruli ($F=9.21$, $p<0.0001$).

The area of the juxtamedullary glomeruli was significantly higher ($F=329.29$, $p<0.0001$) than that of superficial glomeruli, independently of the different methods of obtaining and processing renal tissue, or the age of the animals.

The results indicate that glomerular area is greater in juxtamedullary glomeruli, increases with age, and that different methods of obtaining and processing the renal tissue do have influence on glomerular size.

Introduction

The total glomerular filtration rate depends upon the structural integrity, number and area of glomeruli. In this context, counting the number of glomeruli as well as measuring glomerular diameter, area or volume is nowadays widely employed in human and experimental biology (*Abdy et al., 1998; Fogo et al., 1990; Hoy et al., 2003; Hughson et al., 2003; Li et al., 2002a; Li et al., 2002b; Merlet-Bénichou et al., 1999; Nyengaard & Bendtsen, 1992; Taal et al., 1998*).

Although functional and morphological differences between superficial and juxtamedullary glomeruli have been described (*Olivetti et al., 1977; Beckwith, 1997; Hoyer et al., 2000*), this is not always taken into consideration when performing morphometric studies on renal pathology (e.g. sampling and number of measured glomeruli); on the other hand, some contradictory results in relation to this point have

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been published (*Newbold et al., 1992; Samuel et al., 2005; Wiltrakis, 1972*).

The aim of this paper was to study, in female rats, the influence of age (2-8 months), location (superficial and yuxtamedullary), specimen preparation techniques (fixed in 4% formaldehyde, snap-frozen in liquid nitrogen, fixed in alcoholic Bouin, fixed by perfusion with 4% paraformaldehyde), on the area of the glomerular tuft. These factors could contribute to explain the inconsistency found between the different numerical values reported by different authors.

Materials and Methods

The design of this study was unpaired, comparative, prospective, experimental and longitudinal.

Wistar female rats of 2, 4, 8 and 10 months (6 animals for each group) from the Bioterio of the Centre of Experimental Pathology, Department of Pathology, School of Medicine, University of Buenos Aires were housed under a 12/12-hour day/night cycle at a steady temperature of 22 ± 2ºC in individual suspended wire-bottomed cages with free access to tap water and food (Purina Meals, Buenos Aires). All conditions for husbandry of animals followed NIH Guidelines for the Care and Use of Laboratory Animals. At the end of the experimental period, the rats were weighed and anesthetized with sodium thiopental (40 mg/kg body weight), renal artery and vein were simultaneously clamped and both kidneys were removed, weighed and sagittaly sectioned. The left kidney was fixed in 4% formaldehyde, the anterior half of the right kidney was snap-frozen in liquid nitrogen and the posterior half fixed in alcoholic Bouin (formol/acetic acid/1% piric acid in alcohol/water; 26:7:45:22 ml; Duboscq-Brasil). Other kidneys from rats of 2 and 10 months (6 animals for each group) were fixed by perfusion with 4% paraformaldheyde in 0.135 mol/L phosphate buffer (*Maunsbach, 1966*). Kidneys collected in liquid nitrogen were cut in a cryostat while the other kidney samples were embedded in paraffin and the obtained sections were stained with hematoxylin-eosin. A total of 36 animals were used.

One sagital section per rat was obtained and the tuft area of each individual glomerular profile was delineated and measured in μm^2 with an image analyzer employing the Image Pro Plus 3.0 Program®. Superficial glomeruli were considered as those located up to 250 μm from the capsule and juxtamedullary glomeruli those located close to the outer stripe of the outer medulla (*The Renal Commission of the International Union of Physiological Sciences, 1988*) near the arcuate arteries. A total of 7778 glomeruli were measured.

Results were analyzed using three-ways analysis of variance (ANOVA) with the statistical software "STATISTICA for Windows" by StatSoft, Inc. (*1999*), employing as dependent variable the glomerular area and as independent variables the localization of glomeruli, the method of processing the renal tissue and the age of the rats.

Results

Histopathological analysis did not show evident microscopic alterations at any age.

The mean ± standard deviation (SD) of the glomerular area, as well as the number of glomeruli measured at different months and according to the different procedures employed, is indicated in Table I.

Glomerular area was larger in kidneys collected in liquid nitrogen than in those fixed in Bouin; These results were obtained in superficial and in juxtamedullary glomeruli ($F=29.60$, $p<0.0001$**).**

Glomerular area increased with age in superficial as well as in juxtamedullary glomeruli ($F=9.21$, $p<0.0001$).

The area of the juxtamedullary glomeruli was significantly higher ($F=329.29$, $p<0.0001$) than that of superficial glomeruli independently of the different methods of obtaining and processing the renal tissue, or the age of the animals.

Discussion

Intrarenal heterogeneity in the population of nephrons is a well-established fact from a functional and morphological point of view (*Olivetti et al., 1977; Hoyer et al., 2000; Jamison, 1973*). This

Table 1. Glomerular area (μm²) by location forms of obtaining and processing renal tissue and age of animals.

	Localization	Month 2		Month 4		Month 8		Month 10	
		n	Mean ± SD*	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Formalde-hyde 4%	Superficial	292	5406,0 ± 324,8	377	5943,2 ± 433,2	439	6564,7 ± 861,2	342	7894 ± 426,5
	Yuxtamedul-lary	364	8209 ± 371,8	496	9473,5 ± 861,3	452	9771,5 ± 773,7	191	13245,9 ± 1407,8
Bouin	Superficial	297	4380,5 ± 256,4	334	5873,7 ± 665,7	323	6894,7 ± 549,4	257	8159 ± 1028,2
	Yuxtamedul-lary	467	5818,2 ± 372,7	398	7418,8 ± 687,7	383	8742,5 ± 730,1	167	11350,4 ± 1092
Nitrogen	Superficial	160	7873,0 ± 789,9	166	9973,7 ± 1289,4	151	9519,4 ± 1301,2	-	-
	Yuxtamedul-lary	248	9145,2 ± 534,5	264	12495,8 ± 976	250	13790,6 ± 1986,8	-	-
Perfusion	Superficial	264	5501,8 ±431,8	-	-	-	-	316	8218,6 ± 1010,9
	Yuxtamedul-lary	214	7979,6 ± 1137,1	-	-	-	-	226	11805,2 ± 1064,3

*SD: standard deviation, p values are indicated in the text.

can explain the preferential localization of certain diseases in specific zones within the kidney, as reported by some authors (*Hoyer et al., 2000; Rich, 1957; Habib & Gubler, 1971; Ikoma et al., 1990)* in human as well as in experimental animals. However Elema and Arens (*1975*) did not find preference of glomerular disease for any specific area of the renal cortex in Wistar rats with spontaneous FSGS (Focal and Segmental Glomerulosclerosis). These facts are not always taken into consideration when studying renal diseases.

Glomerular mass would be a very important factor in the pathophysiology of renal diseases (*Brenner & Mackenzie, 1997; Luyckx & Brenner, 2005*). This explains the different types of studies designed to evaluate the number and size of glomeruli in physiological and pathological conditions, as well as the importance in the study of the different methodologies employed to carry on these types of studies.

In this experiment no evident microscopic alterations were observed in the kidneys of the rats killed at different periods; these results are similar to those observed previously (*Elema & Arens, 1975*) in female normal rats of similar ages.

On the other hand, laboratories differ from one another in their way of fixing and processing the renal tissue in experimental animals or renal biopsies, and these facts should be taken into consideration when comparing the results obtained by different researchers. Few authors have systematically studied the influence of these factors, as we and Miller and Meyer (*1990*) have.

We measured all the glomeruli found in the sections, since we did not find usually employed validated standardized procedures for the adequate number of glomeruli to be measured in experimental animals.

The method of obtaining and processing the renal tissue influences the results to be obtained. The greater glomerular area was found in kidneys "snap-frozen" and cut in a cryostat, followed by those fixed

by perfusion. The influence of different procedures has been studied by Miller and Meyer (*1990*) that found that the glomerular size was larger on those fixed by perfusion in relation to those fixed by immersion.

It is common practice to measure glomerular area without taking into consideration the difference between superficial and juxtamedullary glomeruli; however, as Pesce (*Pesce, 1998*) says "if a difference in size does exist, then each compartment should be sampled separately"

It is also clear that the number of glomeruli to be analyzed should vary if glomeruli of both areas of the cortex are measured together or, on the contrary, glomeruli of each area are measured separately.

We found that juxtamedullary glomeruli were larger independently of the age of the animals, and also found an increase in glomerular size along with the age of the rats independently of the method employed to process the renal tissue.

Iversen et al. found that glomerular diameter was larger in juxtamedullary glomeruli at seventy weeks but not at ten weeks in Wistar-Kyoto rats and spontaneously hypertensive rats (*Iversen et al., 1998*).

Cortes et al. (*Cortes et al., 1992*) described greater inner glomeruli in rats and a sevenfold increase in size between 5 weeks and 2 years of age, while human glomeruli increased sevenfold in size from infancy to adulthood and then declined during senescence.

In a study carried out in children less than 16 years of age, it has been reported that glomerular size increases with age; on the other hand the authors reported that glomeruli in the inner cortex were larger than those in the middle and outer cortex (*Moore et al., 1993*).

Li et al (*2002*) described in human beings an increase in glomerular size between infancy and adolescence, but later on the growing was slower; there was no difference between females and males. The mechanisms of increase in glomerular size could be different.

The aim of this paper was to study, during the 10 first months of the life of female rats, if the area of juxtamedullary glomeruli was larger than the area of superficial glomeruli, the glomerular size at different ages; and to evaluate the influence of different fixatives and methods of obtaining the renal tissue in relation to glomerular area.

The results obtained indicate that glomerular area is greater in juxtamedullary glomeruli, that glomerular area increases with age, and that different methods of obtaining and processing renal tissue do have an influence on glomerular size. Fixation by perfusion seems to be the best option. These facts should be taken into consideration when measuring and comparing glomerular area.

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