Ontogeny of Single Nephron Filtration Distribution in Canine Puppies

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Summary

The ontogeny of single nephron filtration distribution was studied in 29 canine puppies two hr to 42 days old using the [¹⁴C] ferrocyanide method as modified by Coehlo (9). The ratio of ¹⁴C counts in outer cortical nephrons (OC) to counts in juxtamedullary nephrons (JM) were plotted against age for each puppy. This ratio (OC:JM) provided an index for comparing the single nephron glomerular filtration between regions. A four-fold increase in OC:JM occurred from two days to four wk. The youngest puppy, two hr old, showed a ratio of zero because no counts were detected in OC at that age.

The most rapid increase occurred in the first 8 days of life. By the fourth wk, the mean OC:JM ratio was 0.72 ± 0.06 , approaching values reported by other laboratories for adult dogs. In contrast to age-related changes in OC:JM ratio, the ratio of middle cortical to JM counts showed no age-related increase. Cortical blood flow distribution was determined by the microsphere method. The ratio of blood flow to outer and inner cortex increased from 0.43 at one-half a day to a mean of 2.93 after one wk. The ratio of OC:JM single nephron filtration to OC/inner cortical nephron renal blood flow was 0.36 from 3 days to 26 days ($n = 12$) and did not change significantly with age, indicating that changes in zonal filtration matched the alterations in cortical blood flow distribution.

Speculation

These studies suggest that although systemic arterial pressure changes probably play a role in the increasing single nephron filtration in the outer cortex of the maturing puppy, it is likely that plasma flow is a governing factor.

In the kidney of the neonate, the juxtamedullary nephrons (JM) are more fully developed than nephrons located in the outer cortical areas; maturation proceeds from the inner cortex (IC) to the outer cortex (OC) (13, 26, 27). A similar centrifugal development has been described for renal blood flow (RBF) distribution (2, 6, 8, 19, 24). In the dog, a relatively greater IC flow is seen at birth and during the first 2 to 3 wk of life. After 3 wk, this pattern of intracortical blood flow distribution is similar to that in the adult dog. By 10 to 12 wk of age, RBF and the cortical/medullary distribution are like adult patterns (8). Available studies suggest that the maturation of filtration may proceed in a centrifugal fashion similar to that described for RBF (3, 11, 12, 30); however, there is less documentation available.

In the rat, Aperia *et al.* (4) noted a sharp increase in superficial single-nephron filtration rate per g kidney weight between 17 and 30 days. Spitzer and Brandis (28) suggested that in the guinea pig during the first two wk of life, glomerular filtration was due mainly to JM. Thereafter, the increase in superficial nephron filtration accounted for most of the increase in total kidney filtration. In the dog, Horster and Valtin (15) reported an increasing contribution of superficial nephron filtration to total kidney

filtration during the first four months of postnatal life. Glomerulogenesis might account for these increases in filtration because it has been demonstrated to continue in the OC for wk after birth in certain species including rats and dogs (16).

For some species, the pattern of glomerular filtration distribution during the first few days of life has been studied by comparing single nephron filtration rate in superficial nephrons with total kidney filtration. In the neonatal dog, single nephron filtration rates in the OC cannot be measured by micropuncture techniques because of the low proximal tubular flow in the first 3 wk of life.

The ontogeny of single nephron filtration distribution was studied in canine puppies two hr to 42 days old using the [¹⁴C] ferrocyanide method (10, 14) as modified by Coehlo *et al.* (9). This method allows a direct quantitation of single nephron filtration in neonatal animals where other methods are technically not feasible. The results indicate that single nephron filtration is greatest in the JM irrespective of age. The ratio of OC to JM filtration is lowest in the newborn period and increases progressively to reach adult levels by the fourth wk of life. The change in filtration distribution with maturation appears to be due in part to the rise in perfusion pressure with age, but intrarenal factors are implicated as well.

MATERIALS AND METHODS

Mongrel puppies, aged 2 hr to 42 days were studied. Puppies remained with the litter until one hr before the experiment. Food intake was controlled by keeping the litter size relatively constant during the wk the siblings were studied. Each mother nursed about three to four siblings.

Prior to the experiment the puppies were weighed, fasted for one hr, and anesthetized with pentobarbital (12 to 22 mg/kg depending upon age). Body temperature was maintained at 37°C on a thermoregulated small animal surgical table. After tracheostomy, one femoral artery was catheterized to the aortic root for the injection of microspheres. The other femoral artery was used to monitor blood pressure. The right external jugular vein was catheterized for infusion of ferrocyanide, and the left jugular vein was catheterized for fluid infusion. Urine was collected from both ureters. Urine and surgical fluid losses were replaced volume for volume with electrolyte solution containing 25 mEq sodium per liter and 20 mEq potassium per liter. The left kidney was carefully exposed retroperitoneally, and the renal vessels were loosely tied. The incision was closed with clamps and kept moist. A period of 60 min was allowed for postsurgical recovery.

Three 10-min urine samples were collected after surgical recovery. The animals then received an infusion of microspheres or [¹⁴C]ferrocyanide or microspheres followed by [¹⁴C]ferrocyanide. Microspheres, $15 \pm 5 \mu\text{m}$ in diameter (New England Nuclear; 0.5 ml ¹⁴¹Ce or 0.3 ml ⁸⁶Sr randomly assigned) were infused for 30 sec for determination of cortical blood flow distribution. [¹⁴C]FeCN 125 to 250 μCi (New England Nuclear, Boston, MA) in 0.5 ml fresh 10% FeCN solution was then infused for 15 to 18 sec during which time arterial blood was sampled for hematocrit and [¹⁴C]

ferrocyanide concentration. No significant change in blood pressure was observed. The left kidney was then immediately ligated, removed, and quick frozen in an acetone dry ice bath. Right kidneys were later removed in all animals for weighing and microsphere analysis.

The frozen left kidney was fractured into two sections using a sharp knife. Rectangular pieces of cortex from each kidney were divided into outer and inner sections in a 2:1 weight ratio. Triplicate samples for each kidney were weighed and counted for gamma activity. The ratio of counts/g of OC to counts/g of IC served as an index of RBF distribution (8).

The remaining left kidney was fragmented, placed into a chilled vial of 20 ml FeCl_3 solution, and stored at -20°C for 19 to 22 hr. The tissue was transferred to 100 ml Erlenmeyer flasks, washed, and macerated in 20% HCl at 41°C for 6 hr. The samples were washed and stored in distilled water at 4°C .

Individual nephrons were teased out with fine needles (Circon Microsurgical) under a remote-controlled, fiber optics-illuminated dissecting microscope (Unislide Velmex A 4000). Each nephron was dissected up to the pars recta (in some cases to the descending loop of Henle). Nephrons dissected from the surface of the kidney were designated as OC. Those closest to the arcuate arteries were designated as JM. The region between represented the middle cortical nephrons (MC). At least 10 nephrons were dissected from each region of each kidney.

Isolated nephrons were placed on glass cover slip fragments and transferred to a Wild Hereburg microscope set up for camera lucida tracing. The nephron was traced, and the dye front was recorded. Prussian blue precipitate appeared in the glomeruli and along the tubule to the dye front. Nephrons were macerated overnight in 0.5 ml nitric acid. Scintillation fluid (2:1 toluene: Triton X-100 solution containing 5 g of Omnifluor from New England Nuclear) was then added, and the vials were counted for beta activity. Each vial contained from three to six nephrons. Only counts more than twice background were accepted; as Kuruda suggests, when radioactivity in dissected nephrons is low, background radiation may make interpretation difficult (23).

The activity in the tubular portions distal to the dye front was judged to represent extraluminal contamination, and this amount was subsequently subtracted from each tubular count. The extraluminal contamination represented 10% of total counts in the JM and 15% in OC. To determine the influence of glomerular size on nephron counts, some nephrons were divided into three portions: glomerulus, tubule proximal to the dye front, and tubule distal to the dye front. Glomerular activity represented 36% of total tubular counts in the OC and 38% in the JM. These data indicate that extraluminal contamination and glomerular tuft contribution were similar in OC and JM.

Fig. 1. Age of animals in days plotted against kidney weight. $r = 0.96$; $P < 0.01$, $Y_c = 0.268 X + 1.80$.

Fig. 2. Age of animals in days is plotted against MAP in mm Hg. $r = 0.73$; $P < 0.01$; $Y_c = 1.43 X + 42.40$.

Statistical analysis was performed using the Student t test for group data and for slopes. Linear regression was determined by the least-squares method. Values are given as mean $\pm$ S.E. $P < 0.05$ was considered significant.

RESULTS

BODY WEIGHT AND KIDNEY WEIGHT

Body weight increased linearly with age. Wet kidney weight also showed a linear increase with age (Fig. 1) ($r = 0.96$; $P < 0.01$; $Y_c = 0.268 X + 1.80$) and remained a constant proportion ($0.80 \pm .01\%$) of body weight throughout the growth period.

MEAN ARTERIAL PRESSURE

There was a 2.6-fold increase in mean arterial blood pressure (MAP) over the first 6 wk of neonatal maturation from 44 mm (3 days) to 116 mm Hg (6 wk) (Fig. 2). As a group ($n = 29$), the rise in pressure was linear with age ($r = 0.73$; $P < 0.01$; $Y_c = 1.43 X + 42.40$). In the first wk of life, MAP was not age related.

DISTRIBUTION OF SINGLE NEPHRON FILTRATION

OC in all puppies studied ($n = 29$) had a mean of 11.6 ± 1.2 cpm whereas JM had a mean of 23.4 ± 2.6 cpm. The ratio of ^{14}C counts in the OC to counts in the JM are plotted against age for each puppy (Fig. 3). This ratio (OC:JM) provides an index for comparing the single nephron glomerular filtration rate (SNGFR) between regions. The youngest puppy, 2 hr old, showed a ratio of zero because no counts were detected in the OC at this early stage. The mean OC:JM in the first 2 days of life was 0.17 ± 0.07 ; by 4 wk of age, the mean was 0.72 ± 0.06 , a four-fold increase. The most rapid increase in OC:JM occurred in the first 7 days of life and plateaued at the second to third wk. A slower rise occurs by the fourth wk, at which time the mean OC:JM ratio was 0.72 ± 0.06 , approaching values reported by other laboratories for adult dogs (9). The regression could best be described as a fourth order polynomial, assuming that an asymptote of 0.8 could be achieved [$Y_c = 0.092 \pm 0.0982X - 8.872 (10^{-3}) X^2 + 3.475 (10^{-4}) X^3 - 4.434 (10^{-6}) X^4$]. In contrast to age-related changes in OC:JM ratio, the ratio of middle cortical to juxtamedullary counts (0.064 ± 0.05) showed no age-related increase. Figure 4 shows OC:JM as a function of MAP ($r = 0.60$; $P < 0.01$; $Y_c = 0.006 X + 0.17$). Urine flow failed to correlate with single nephron glomerular filtration (SNGF) in our studies.

DISTRIBUTION OF CORTICAL BLOOD FLOW

Studies were performed in 17 animals one-half day to 22 days old. Blood flow per g of tissue was higher in OC in 83% of cases

Despite the fact that single nephron filtration was consistently higher in JM. The ratio of counts/g of OC to counts/g of IC increased from 0.43 at one-half day to 1.43 ± 0.22 ($n = 11$) in the first wk of life to 2.93 ± 1.04 ($n = 6$) beyond one wk ($P < 0.05$). The increase of this ratio with age can be described as a linear function $r = 0.77$; $P < 0.01$; $Y_c = 0.24 X + 0.29$. The fewer numbers of puppies beyond one wk of age limited our comparing slopes of OC:JM SNGFR and OC:IC RBF.

DISTRIBUTION OF RBF VERSUS DISTRIBUTION OF SINGLE NEPHRON FILTRATION

In 9 puppies, both blood flow and single nephron filtration were measured in the same animal. Eight animals were 2 to 9 days old; one was 22 days old. Because single glomerular blood flow was not determined, values for filtration fraction per nephron are not available. The ratio of OC:JM SNGF to OC:IC RBF, however, may serve as an index of this function. This ratio of 0.36 ± 0.06 (S.E.) did not change significantly with age ($r = 0.28$; $P > 0.05$), indicating that the changes in zonal filtration matched the alterations in cortical blood flow distribution.

DISCUSSION

These studies directly demonstrate for the first time a centrifugal pattern of maturation of glomerular filtration in the immediate neonatal period. In the first 3 days of postnatal life, the filtration rate of JM was four and one-half times greater than that in the outermost cortical nephrons. The greatest increase in filtration rate of outer nephrons occurred in the first wk of life. Thereafter,

Fig. 3. Age of animals in days is plotted against the ratio of OC to JM SNGF. $r = 0.81$; $P < 0.01$; $Y_c = 0.02 X + 0.27$; $n = 29$. This ratio was determined by comparing the ^{113}C ferrocyanide counts in dissected OC and JM.

Fig. 4. OC:JM SNGF is plotted against MAP. $r = 0.60$; $P < 0.01$; $Y_c = 0.006 X + 0.17$.

a slower rise of OC:JM ratio occurred, and by the fourth wk, values similar to those reported for adult dogs were achieved.

A finely graded change in SNGFR from one cortical region to the adjacent concentric region, if verified, would support the view of an infinitely partitioned centrifugal distribution of filtration function as postulated by Spitzer and Brandis (28). A comparison of such adjacent regions with nearly the same SNGFR would presumably turn up SNGFR ratios that were nearly equal to one. Only in nonadjacent regions, such as OC and IC would the regional heterogeneity be significant enough to give a ratio different from one.

We failed to show a difference between either cortical extreme and the adjacent MC as shown by the ratios OC:JM and MC:JM. If the middle region were indeed heterogeneous, then sampling error could mask differences by cancelling out higher counts from nephrons randomly sampled near the juxtamedullary region with lower counts likewise obtained near the OC. Hence, the MC would appear to be homogenous due to this sampling. An alternate explanation may be that the outer and juxtamedullary cortical regions define two distinct and unique populations. A heterogeneous intermediate region would contain elements of both regions.

The greater increase in OC over JM SNGF with age may be due to any of the factors which can affect filtration. These include glomerular surface area and permeability, hydrostatic forces, and renal plasma flow. It is unlikely that there is a greater increase in glomerular capillary surface area in OC compared to IC glomeruli because John (18) has shown that the ratio of OC to IC glomerular capillary surface area decreases at three wk, at a time when OC/JM SNGF is increasing.

Changes in glomerular permeability coefficient with age have been evaluated in humans and rats (5, 7). In the human, Arturson *et al.* (5), using dextran tracers, suggested that glomerular permeability increased with age. An increase in glomerular permeability with age has also been reported in Munich-Wistar rats (29). Recently, however, in the same species, Ichikawa and Brenner (17) suggested that glomerular ultrafiltration coefficient is not a factor limiting SNGFR in the neonatal rat because filtration equilibrium exists. However, because filtration equilibrium has not been definitely shown in dogs, this possibility must be considered.

Factors determining glomerular filtration rate in maturing Munich-Wistar rats have recently been studied by micropuncture techniques (17, 29). Between 40 and 140 days of life, although afferent and efferent arteriolar resistances were higher in the younger rats, glomerular capsular pressure was unchanged. Because proximal tubular hydrostatic pressure was also constant during growth, it was concluded that changes in hydrostatic forces did not contribute to the increase in SNGFR with growth. However, it should be noted that in these rats systemic arterial pressure was also constant during the period of study. Allison *et al.* (1) studied rats less than 30 days old and reported that glomerular capillary pressure (sum of stop flow tubular pressure and arteriolar oncotic pressure) was lower than in adult rats. Studies in the neonatal guinea pig (12 hr to 49 days) have also shown that glomerular capsular pressure (indirect method) increased with age (28).

In dogs during the first three wk of life, an increase in mean systemic arterial pressure occurs, and positive correlation between glomerular filtration rate and systemic arterial pressure has been demonstrated (15, 20). In our studies in the first wk of life, OC:JM SNGF increased significantly, whereas MAP remained constant. Thereafter, age-related rises in both OC:JM SNGF and MAP were noted. When OC:JM was plotted as a function of mean systemic pressure, a regression of 0.60 was obtained. Such a positive correlation indicates that the centrifugal pattern of development of SNGFR could be related in part to the rise in mean systemic pressure. As shown in Allison's study (1), although arterial blood pressure was correlated with SNGFR, it did not correlate with hydrostatic pressure in efferent arterioles, pointing out the need for direct measurements of glomerular dynamics if firm correlations are to be drawn.

Current data indicate absence of filtration equilibrium in the adult dog with normal perfusion pressures (22, 25). If this is the case in the puppy as well, then changes in age-related permeability may well play an important role in the increasing SNGFR noted. However, in adult dogs, when perfusion pressure is diminished, filtration equilibrium becomes evident (21). Inasmuch as we are dealing with a low-pressure system in the puppy, it is possible that filtration equilibrium exists in this state.

In our studies, the changes in RBF distribution with age parallel the changes in zonal SNGFR. The centrifugal maturation of renal cortical blood flow has previously been documented by Jose *et al.* (19), Olbing *et al.* (24), and Aschinberg *et al.* (6). Although filtration fraction per nephron could not be calculated from these studies, the ratio of SNGFR OC:JM to OC:IC flow remained constant with age. Thus, the changes in SNGFR distribution with age may be secondary to concomitant alterations in cortical blood flow distribution and increases in glomerular plasma flow. Aperia and Herin (4), Ichikawa and Brenner (15) and Tucker and Blantz (29) have previously indicated the importance of plasma flow in dictating changes in glomerular filtration in the maturing rat.

In conclusion, these studies suggest that although systemic arterial pressure changes probably play a role in the increasing SNGFR noted with maturation in the puppy, it is likely that plasma flow is a governing factor, and a role for changes in permeability cannot currently be excluded.

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