Cortical and Medullary Canine Renal Lymph Formation during Acetylcholine Induced Renal Vasodilation *

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It is widely held that both cortex and medulla of the mammalian kidney participate in renal lymph formation (1). The influence of the renal cortex on renal lymph composition is demonstrated by renal lymph to arterial plasma ratios less than unity for inulin (2), creatinine (3) and p-aminohippurate (PAH) (4). The finding that cortical extraction of inulin is reflected in the renal lymph concentration of that substance promoted the hypothesis that renal lymph may be derived from both blood plasma and tubular reabsorbate (2). More detailed information concerning the mechanisms of cortical renal lymph formation requires further experimentation.

Lymph formation in the renal medulla is well supported by anatomical findings in the human kidney (5, 6). Even so, it has not yet been possible to demonstrate changes in canine renal lymph composition when medullary function is altered (3, 7). Thus, additional data are needed to establish the importance of the medulla in renal lymph formation.

The experiments of the present study are based on the finding that the capsular lymphatic vessels of the canine kidney drain primarily the cortex, while the hilar lymphatic vessels are believed to drain both cortex and medulla (8). The avid cortical extraction of PAH and the absence of protein from tubular reabsorbate make these two substances of particular interest. Additional information concerning both cortical and medullary renal lymph can thus be gained by determining capsular and hilar renal lymph concentrations of PAH and protein before and during changes in renal hemodynamics such as those produced by acetylcholine (ACh).

Methods

Mongrel dogs (20–30 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and the left kidney exposed through a flank incision. The ureter was catheterized for collecting urine, and a catheter was placed into the renal vein via the gonadal vein for collecting renal venous blood. Capsular and/or hilar renal lymphatic vessels were catheterized as previously described (4, 9). The femoral artery and vein were

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catheterized to facilitate collection of arterial blood samples and administration of infusions, respectively. All animals received an infusion of 0.9% NaCl intravenously at 2 ml/min for the duration of the experiment. PAH was added to the infusion to maintain constant plasma levels of this substance. Sufficient sodium heparin was administered to each animal to prevent clotting of blood and lymph.

Renal arterial infusion was accomplished using a Harvard syringe infusion pump delivering 0.1 ml/min via a 20 guage needle inserted through the wall of the renal artery. The infusion consisted of normal saline solution during each control period and acetylcholine chloride in saline (0.01 mg/min/kg body weight) during each experimental period. Lymph collections during the experimental period were not begun until urine flow had stabilized (20–30 min).

Lymph was collected under mineral oil. Arterial and renal venous blood samples were drawn at equal intervals of not more than 30 minutes throughout each lymph collection.

Concentrations of protein and PAH in renal lymph, arterial plasma and renal vein plasma were determined. Protein concentrations were obtained using a Biuret method, and PAH concentration by the method of *Smith* et al. (10). Urinary concentrations of PAH were obtained in order to calculate plasma clearance of this substance as an estimate of renal plasma flow.

Arterial blood pressure was monitored continuously to assure that the experimental infusion did not alter this parameter. Further, data from each experiment was carefully examined to make certain that Tm PAH had not been exceeded during the acetylcholine infusion.

Statistical analysis used in the present study consisted of the Student's test suitable for paired data.

Results

Mean values for fluid dynamic factors measured in the present study are shown in Table 1 along with mean PAH extractions. Renal arterial infusion of ACh caused significant changes ($P < .05$) in renal perfusion, urine flow and PAH extraction. These findings are consistant with those of other investigators (11). It should be noted, however,

Table 1 PAH extraction and flows of lymph, plasma and urine before and during close arterial infusion of acetylcholine (ACh).

	Control			ACh		
	Mean	S.E.	n	Mean	S.E.	n
Capsular Lymph Flow, μL/min	13.5	4.7	6	28.5	6.9	6
Hilar Lymph Flow, μL/min	20.3	2.2	7	30.1	6.0	7
Renal Plasma Flow, ml/min	185.7	13.6	13	286.2	28.2	13
PAH Extraction, Percent	67.5	1.4	13	57.5	2.3	13
Urine Flow ml/min	0.25	0.03	13	1.99	0.25	13

that the mean flow rates for both capsular and hilar renal lymph were greater during ACh infusion than during the control periods. The increase in capsular renal lymph flow was statistically significant ($P < .05$) while that for hilar lymph was not ($P < .1$).

Concentrations of capsular and hilar renal lymph protein are related to those of arterial blood plasma in Table 2. It is of interest that ACh infusion caused a significant increase in capsular renal lymph flow (Table 1) but no change in the lymph to arterial plasma ratios (L/P_A) for either capsular or hilar lymph protein.

Table 2 Renal lymph to arterial plasma ratios (L/P_A) for protein before and during close arterial infusion of acetylcholine (ACh).

	Capsular Lymph			Hilar Lymph		
	L/P_A Mean	S.E.	n	L/P_A Mean	S.E.	n
Control	0.33	0.06	6	0.33	0.03	7
ACh	0.31	0.05	6	0.33	0.03	7

Mean concentrations of PAH found in capsular renal lymph (n = 6), hilar renal lymph (n = 7), arterial blood plasma (n = 13) and venous blood plasma (n = 13) before and during close arterial infusion of ACh are presented in Fig. 1. Close arterial infusion of ACh caused no change in capsular lymph and arterial blood plasma concentrations of PAH. In contrast, the PAH concentrations of both hilar renal lymph and renal venous plasma were significantly greater during ACh infusion than during the control period $P < .05$).

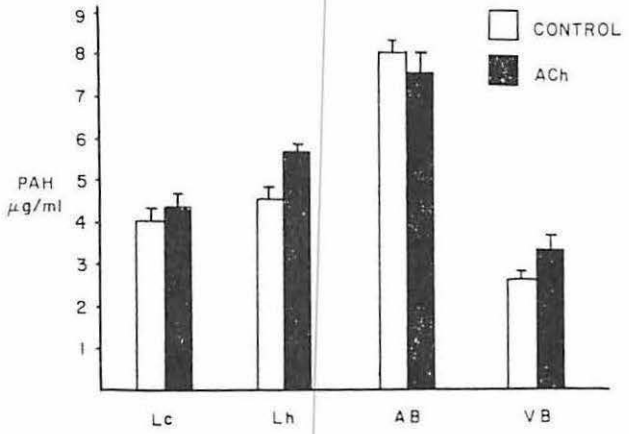

Fig. 1 Concentrations of p-aminohippuric acid (PAH) in capsular renal lymph (Lc), hilar renal lymph (Lh), arterial blood plasma (AB) and renal venous blood plasma (VB). Means and standard errors are shown for samples collected during control periods and during renal arterial infusion of acetylcholine (ACh).

Discussion

The experiments of the present study show that renal arterial infusion of acetylcholine is accompanied by increased renal lymph flow as well as increased renal blood flow and urine flow. It is known that increased urine flow alone does not cause an increase in renal lymph flow (12). Conversely, it has been shown that renal lymph pressure increases with increased renal blood flow in the pump-perfused kidney (13). Thus the lymph flow increases observed in the present study appear to be due primarily to the increased renal blood flow during ACh infusion. It is of some interest that the capsular lymph response to increased renal blood flow was somewhat greater than that of hilar lymph, especially since capsular and hilar lymphatic vessels are known to communicate through the interlobular plexus of lymph capillaries (14, 15). A less dramatic hilar lymph flow response may result from shunting of lymph through parallel hilar channels arising from the extensive lymphatic plexus in the area of the arcuate blood vessels (14, 15). However, there is little chance for such shunting of the capsular lymph since the capsular lymphatic vessels apparently drain directly from the interlobular lymph capillary networks with little direct intercommunication (14, 15).

In contrast to the conclusions of earlier workers (8), the present investigation verifies the previous observation that renal lymph flow may increase without a concomitant decrease in lymph protein concentration (16, 17). This observation constitutes one of the major differences between lymph formation in the kidney and the dog's leg (18). The data of the present study is thus consistent with the hypothesis that a portion of renal lymph may be formed as a filtrate of the interlobular veins (9, 16).

Several studies have now been published which indicate that the capsular lymphatic vessels of the canine kidney primarily drain the cortex while those of the hilus may serve to drain both cortex and medulla (8). Since PAH is avidly extracted in the renal cortex, it is not surprising that the capsular lymph PAH concentration is significantly less than that of arterial plasma. PAH is not extracted in the renal medulla, thus hilar lymph might be expected to have a PAH concentration greater than that of capsular lymph, the difference being determined by the ratio of cortical to medullary lymph in the hilar sample. Mean capsular and hilar lymph PAH concentrations found in control periods of the present study were similar, and approximately one half that of arterial plasma. These findings are in agreement with the capsular and hilar lymph PAH data of *Cockett* et al. (19), and suggest that the ratios of cortical to medullary lymph in the control hilar samples were sufficiently great to render the medullary component undetectable.

Very low rates of medullary lymph formation are consistent with the hypothesis that movement of vascular water in the renal medulla is primarily from descending vasa recta to ascending vasa recta (20). Although medullary lymph formation may occur continuously in the normal kidney (1), its rate of formation is apparently very low during usual experimental conditions (20).

In contrast to findings of the control periods, the hilar renal lymph PAH concentration obtained in the present study increased in parallel to that of venous plasma during renal arterial infusion of ACh, while that of capsular lymph did not. ACh is known to cause an overall renal vasodilation involving both cortex and medulla (11). Since increased cortical blood flow will result in a similar increase in the rate of PAH secretion (11),

a stable capsular lymph PAH concentration may be expected. The increased hilar lymph PAH concentration, however, suggests that the increased medullary blood flow resulted in significantly increased vasa recta filtration and lymph production. The study published by *Carone* et al. (20) suggests that medullary hemodynamics may be of such a nature that fluid uptake by ascending vasa recta largely balances filtration by the descending vasa recta. The data of the present study suggest that the sudden blood flow increase which accompanies ACh infusion changes medullary hemodynamics in favor of filtration.

Summary

Canine capsular and hilar renal lymph flow, protein concentrations and PAH concentrations were studied before and during renal arterial infusion of acetylcholine. Acetylcholine caused increased renal blood flow and urine flow which were accompanied by increased renal lymph flow, with no change in lymph protein concentration. As judged by PAH concentration, a medullary component could be identified in hilar, but not capsular, renal lymph during close arterial infusion of acetylcholine. No medullary component could be detected in either capsular or hilar lymph during control periods.

References

1 *Mayerson, H. S.:* The physiologic importance of lymph. In: Handbook of Physiology, Sec. 2, Vol. 2, Circulation, edited by W. F. Hamilton and P. Dow. Washington, D. C. Amer. Physiol. Soc. (1963), 1035-1073

2 *Kaplan, A., M. Friedman, H. E. Kruger:* Observations concerning the origin of renal lymph. Amer. J. Physiol. 138 (1942), 553-556

3 *Santos-Martini, J., E. E. Selkurt:* Renal lymph and its relationship to the countercurrent multiplier system of the kidney. Amer. J. Physiol. 216 (1969), 1548-1555

4 *Keyl, M. J., J.B. Scott, J. M. Dabney, F. J. Haddy, R. B. Harvey, R. D. Bell, H. E. Ginn:* Composition of canine renal hilar lymph. Amer. J. Physiol. 209 (1965), 1031-1033

5 *Rawson, A. J.:* Distribution of the lymphatics of the human kidney as shown in a case of carcinomatous permiation. Arch. Path. 47 (1949), 283-292

6 *Rodin, J. A. G.:* Fine structure of the peribubular capillaries of the human kidney. In: Progress in Pyelonephritis, edited by E. H. Kass. (International Symposium on Pyelonephritis, 2d). Philadelphia, Pa., Davis, 1965

7 *Bell, R. D., M. J. Keyl, F. R. Shrader:* Effects of renal medullary concentrating ability on canine renal lymph composition. Amer. J. Physiol. 216 (1969), 704-706

8 *Sugarman, J., M. Friedman, E. Barret, T. Addis:* The distribution, flow, protein and urea content of renal lymph. Amer. J. Physiol. 138 (1942), 108-112

9 *Bell, R. D., R. D. Ornitz, R. Trautman, I. L. Anderson, M. J. Keyl:* The significance of the renal pelvis and intrarenal veins in renal lymph formation. Invest. Urol. (in press)

10 *Smith, H. W., N. Finkelstein, L. Aliminosa, B. Crawford, M. Graber:* The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. clin. Invest. 24 (1945), 388-404

11 *Harvey, R. B.:* Effects of acetylcholine infused into renal artery of dogs. Amer. J. Physiol. 211 (1966), 487-492

12 *LeBrie, S. J.:* Renal lymph and osmotic diuresis. Amer. J. Physiol. 215 (1968), 116-123

13 *Gazitua, S., J. B. Scott, T. E. Emerson, F. J. Haddy:* Deep venous and lymphatic vessel pressures during renal autoregulation in the in situ dog kidney. Proc. Soc. exp. Biol. Med. 131 (1969), 642-645

14 *Pierce, E. C.:* Renal lymphatics. Anat. Rec. 90 (1944), 315-329

15 *Bell, R. D., M. J. Keyl, F. R. Shrader, E. W. Jones, L. P. Henry:* Renal lymphatics: The internal distribution. Nephron. 5 (1968), 454-463

16 *Bell, R. D., M. J. Keyl, W. L. Parry:* Experimental study of sites of lymph formation in the canine kidney. Invest. Urol. 8 (1970), 356-362

17 *Haddy, F. J., J. Scott, M. Fleishman, D. Emanuel:* Effect of change in renal venous pressure upon renal vascular resistance, urine and lymph flow rates. Amer. J. Physiol. 195 (1958), 79-110

18 *Drinker, C. K., J. M. Yoffey:* Lymphatics, lymph and lymphoid tissue. Howard, Cambridge 1941

19 *Cockett, A. T. K., A. P. Roberts, R. S. Moore:* Renal lymphatic transport of fluid and solutes. Invest. Urol. 7 (1969), 10-14

20 *Carone, F. A., B. A. Everett, N. J. Blondeal, J. Stolarcyzk:* Renal localization of albumin and its function in the concentrating mechanism. Amer. J. Physiol. 212 (1967), 387-393

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