Lymphology 16 (1983) 228–232

Canine Renal Lymph Formation during Acute ECF Expansion

R.D. Bell, A. Lowsitisukdi

Department of Physiology, Chicago College of Osteopathic Medicine, Chicago/Ill.

Summary

Renal lymph flow, composition and pressure were related to renal function and hemodynamics before and during acute extracellular fluid (ECF) expansion (Ringer's solution, 10% of body wt.) in anesthetized dogs. ECF expansion caused increases in renal lymph pressure and flow and a decrease in the plasma concentration and L/P ratio for protein without altering the transfer rate of protein from blood to lymph (lymph clearance). In contrast, the L/P ratios for creatinine and PAH were unchanged following ECF expansion while the lymph clearances of these substances increased roughly in proportion to the increase in lymph flow. These findings are consistent with two alternative hypotheses: a) renal lymph merely participates in the generalized increase in lymph formation that follows nononcotic ECF dilution, or b) some or all of the observed lymph flow increase was derived directly from an excess of tubular reabsorbate.

Introduction

The protein which leaves the kidney in renal lymph is almost certainly derived from blood plasma. As early as 1931, it was inferred that at least some of the renal capillaries leak protein under normal conditions (1). This concept has been generally supported by recent investigators as well (2, 3). In addition, there is ample evidence that some renal lymph may be derived directly from tubular reabsorbate. In 1942 *Kaplan* et al. (4) demonstrated that renal lymph contains significantly less inulin than concurrently collected cervical lymph. This finding yielded the conclusion that renal lymph contains a component derived from inulin-free tubular reabsorbate. This view is supported by electronmicrograph studies that show lymph capillaries in the immediate vicinity of renal tubules (5). These observations were placed on a quantitative basis by *O'Morchoe* and *Albertine* (6) who reported that 13% of cortical lymphatic capillaries were primarily related to tubules. Certainly, tubular reabsorbate appears to have immediate access to lymphatic capillaries, but direct evidence for a reabsorbate component in renal lymph has not yet been obtained. For instance, it has been shown that renal lymph concentrations of inulin, creatinine and PAH are normally greater than those of renal vein blood plasma (3, 7). Thus, it is evident that renal lymph composition may be altered by renal function, but not necessarily by the direct addition of reabsorbate. In addition, significant increases in renal vein pressure would be expected to be transmitted to the peritubular capillaries and oppose uptake of tubular reabsorbate. Under these conditions, protein-free reabsorbate should flood the lymphatic system to increase renal lymph flow while decreasing lymph protein concentration. While elevations in renal venous pressure produce dramatic increases in lymph flow, these procedures are usually without effect on renal lymph protein concentration (2, 7). Finally, it has been shown that renal lymph formation continues in the absence of renal perfusion, if renal small vein pressure is maintained within normal limits (8), thus casting doubt upon the importance of tubular reabsorption to lymph formation.

It is likely that only a portion of renal lymph is derived as a filtrate of postglomerular blood vessels (6, 9). Even so, it has proved difficult to demonstrate other sources of renal lymph. In order to gain additional information, the experiments of the present investigation were designed to study renal lymph formation before and during acute extracellular expansion.

Methods

The left kidney was exposed in five mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg). Catheters were secured in the ureter, femoral artery, vena cava via the femoral vein and cephalic vein. A polyethylene catheter was tied into a hilar renal lymphatic vessel for lymph collections. Renal venous blood collections were made using a catheter inserted into the renal vein via the gonadal vein. Arterial and vena cava pressures were constantly monitored using resistance bridge transducers. Timed collections of urine and renal lymph were made in calibrated containers and lymph was collected under mineral oil to prevent evaporation. Urinary fluid loss was replaced by I.V. injections of Ringer's solution in volumes of 20 ml or less. Each animal received a priming dose of PAH and creatinine followed by a sustaining infusion of 0.9 % saline at 2 ml/min. Sufficient PAH and creatinine was added to the infusion to maintain adequate plasma levels of these substances. In each experiment, a single control sample of renal lymph and urine was collected, for no more than 90 min, with blood sample collections equally spaced by intervals no greater than 30 minutes. After collecting control samples of lymph, plasma and urine, the animals received an I.V. infusion of creatinine and PAH free Ringer's solution (10 % of body wt. given over 90 min). Following a 15 min equilibration period, three consecutive 40 min post-infusion collection periods were taken. Nine additional experiments were conducted as described above, except that capsular renal lymph and renal small vein pressures (SVP) were monitored before and during acute ECF expansion. Renal lymph pressure was obtained using a pressure transducer attached to a polyethylene tube tightly wedged into a capsular lymphatic vessel and small vein pressure was similarly obtained from a catheter inserted through the wall of the renal vein and advanced into the small veins of the cortex (10). Pressures were monitored during a single 20 minute control clearance period and during three additional 20 minute periods following ECF expansion. Lymph, plasma and urine samples were analyzed for creatinine by the Jaffe reaction, and PAH by the method of *Smith* et al. (11). Lymph and plasma protein concentrations were obtained using a biuret reaction. Renal blood flow (RBF) was calculated by the Fick method using PAH and hematocrit data. GFR was estimated by creatinine clearance (Ccr), and whole kidney fluid reabsorption rate (FRR) was calculated as GFR minus urine flow. Lymph clearances (LC) were calculated as (Lymph conc./Plasma conc.) X Lymph Flow, and lymph concentrations were presented as renal lymph to arterial blood plasma concentration ratios (L/P). Prevenous resistance was calculated as (AP-SVP)/RBF, and venous resistance as (SVP-RVP)/RBF in which:

AP = mean arterial pressure,
SVP = renal small vein pressure,
RVP = renal vein pressure, and
RBF = renal blood flow.

Preliminary calculations, using analysis of variance, showed no difference among post-ECF expansion data points. Thus, the data are presented as control v.s. ECF expansion mean values. Tests of null hypotheses were based on Student's t for paired data with $P < 0.05$ considered "statistically significant".

Results

As shown in Table 1, acute ECF expansion was accompanied by significant increases in GFR, urine flow, RBF, SVP and FRR. Significant increases were also obtained in hilar renal lymph flow and capsular renal lymph pressure (Table 2). As seen in Table 2, the L/P ratio for protein decreased following Ringer's infusion, but those for creatinine and PAH were unchanged. The reverse was true for lymph clearance, i.e., the lymph clearance of protein was unchanged, while those for creatinine and PAH were increased. Ringer's infusion resulted in decreases of approximately 20 % in plasma protein, creatinine and PAH concentrations. There were similar decrease in renal lymph creatinine and PAH concentrations, but a disproportionate decrease in renal lymph protein concentration was observed, as indicated by the decreased protein L/P. Femoral artery and vena cava pressures were stable throughout these experiments. Thus, the increase in

Table 1 Renal responses to acute ECF expansion

		Control	ECF expansion
GFR	Mean	42.8	56.5*
ml/min/m^2	S.E.	2.9	3.2
Urine flow	Mean	0.23	4.81*
ml/min (n=14)	S.E.	0.03	0.71
RBF	Mean	3.73	4.95*
ml/min/g	S.E.	0.32	0.42
SVP**	Mean	18.0	25.3*
mmHg (n=9)	S.E.	1.7	1.5
FRR$^+$	Mean	39.0	47.2*
mmHg (n=14)	S.E.	3.2	3.5

*Statistically significant difference ($P < 0.05$)
**Renal small vein pressure
$^+$ Whole kidney fluid reabsorption rate

Table 2 Renal lymph response to acute ECF expansion

		Control	ECF expansion
Hilar lymph	Mean	14.6	24.0*
flow μL/min	S.E.	1.9	2.7
(n = 5)			
Capsular lymph	Mean	6.9	14.8*
pressure mmHg	S.E.	1.2	1.9
(n = 9)			
L/P protein	Mean	0.56	0.34*
(n = 5)	S.E.	0.10	0.02
L/P creatinine	Mean	0.85	0.87
(n = 5)	S.E.	0.04	0.13
L/P PAH	Mean	0.58	0.54
(n = 5)	S.E.	0.06	0.06
LC protein	Mean	8.32	8.31
μL/min (n = 5)	S.E.	1.73	1.20
LC creatinine	Mean	12.20	18.99*
μL/min (n = 5)	S.E.	1.42	0.93
LC PAH	Mean	8.59	12.78*
μL/min (n = 5)	S.E.	1.68	1.76

*Statistically significant difference ($P < 0.05$)

RBF shown in Table 1 was due to a decrease in overall renal vascular resistance. This resistance change is shown to occure entirely in the intrarenal vascular segments proximal to the tip of the SVP catheter (Table 3).

Table 3 Renal resistance (ml/min/mmHg) before and following acute ECF expansion (n = 7)

		Control	ECF expansion
Total	$\bar{x}$	0.67	0.46*
	S.E.	0.09	0.05
Prevenous	$\bar{x}$	0.59	0.37*
	S.E.	0.09	0.04
Venous	$\bar{x}$	0.08	0.09
	S.E.	0.01	0.01

*Statistically significant ($P < 0.05$)

Discussion

It has been determined in previous studies (9) that the renal small vein and lymph pressures rise in proportion to RBF. It should be noted however, that while renal lymph flow may double during large increases in RBF, the L/P ratio for protein remains relatively constant (12). This finding has also been documented following lymph flow increased due to elevation of renal vein pressure (2, 7). In those experiments the increases in filtration of water and protein were proportional to the changes in hydrostatic pressure. Thus, the microvascular permeability, as defined by *Staub* (13), remains unaltered even when renal vein pressure is increased to 40 mmHg (12). Acute ECF expansion resulted in a significant increase in RBF, as expected (16). The increases in SVP and lymph flow and pressure were likewise unremarkable. It is, however, remarkable that the rate of protein transfer from plasma to lymph (LC protein) was unchanged. On the other hand, the SVP increase was small the mean SVP following ECF expansion being still within the normal range found in the dog (10, 12). In any case, acute ECF expansion is shown to result in a more rapid formation of relatively protein-poor lymph without changes in L/P creatinine and PAH. There are two possible mechanisms that could produce this response. Either the lymph flow is increased because of

(a) the addition of greater quantities of protein-free tubular reabsorbate, or (b) more rapid postglomerular filtration of a relatively protein poor fluid.

Addition of tubular reabsorbate. If the lymph flow increase were due to addition of tubular reabsorbate, then we must yet account for the increased lymph clearances of creatinine and PAH. While the added fluid would be creatinine and PAH free, the lymph must still pass through the interlobular and arcuate lymphatic vessels before leaving the kidney (6). During this passage, the lymph would almost certainly come into diffusion equilibrium with the interstitial fluid of the intrarenal connective tissue, particularly with respect to small molecular wt. substances. Thus, irrespective of additions or deletions of reabsorbate, the lymph PAH and creatinine concentrations will most likely reflect those of the intrarenal connective tissue interstitial fluid. The high molecular wt. of the proteins would argue against a significant diffusional exchange of these substances across the lymphatic wall. This suggests that almost all of the protein in lymph is that present in the original vascular filtrate portion of the lymph. This is not to say that a lymphatic transmural protein concentration difference at the interlobular and arcuate levels would not alter lymph flow and protein concentration. Any oncotic pressure difference across the lymphatic endothelium will cause a proportional exchange of water as long as there is adequate time for the diffusional exchange to occur. The protein-free water gained from increased tubular reabsorption will be lost, in part, to the intrarenal connective tissue interstitial fluid – a process which will minimize loss of creatinine, PAH and water from lymph, as well as decrease the steady state interstitial fluid protein concentration. Still, it is possible that free exchange of fluid through the lymphatic endothelial wall may occur, even in the larger intrarenal lymphatic vessels (5, 14), but the implications of this likelihood are yet to be determined.

Accelerated filtration. A second explanation for the data presented derives from the well known relationship of capillary fluid transudation to blood plasma oncotic pressure (15). Microvascular filtration is almost certainly a function of the transendothelial hydrostatic and oncotic pressures operative at any given time (15). We have shown that acute ECF expansion results in an increase in renal small vein pressure as well as a decrease in blood plasma protein concentration. Providing that the renal microvasculature responds to these factors as other tissues do (15), a noticeable increase in renal microvascular filtration and lymph flow, as reported here, should result even if additional tubular reabsorbate were not added. The drop in protein L/P ratio observed can be predicted on the basis of the non-linear relationship between protein concentration and oncotoic pressure (16, 17).

Conclusions

The data derived in the present study are compatible with the hypothesis that renal lymph merely participates in the generalized increase in lymph formation that follows non-oncotic ECF dilution (16, 17). While we favor this explanation, the data are also consistent with the conclusion that some or all of the observed lymph flow increase was derived directly from an excess of tubular reabsorbate; i.e., the data presented do not distinguish between these two possible alterantives.

Acknowledgements

This work was supported by a Grant-In-Aid from the Chicago Heart Association and by the Chicago College of Osteopathic Medicine

References

1 *Drinker, C.K., M.E. Field:* The protein content of mammalian lymph and the relation of lymph to tissue fluid. J. Physiol. 79 (1931) 32–39
2 *Vogel, G., K. Gartner, M. Ulbrich:* The flow rate and macromolecule content of hilar lymph from the rabbit's kidney under conditions of renal venous pressure elevation and restriction of renal function-studies on the origin of renal lymph. Lymphology. 3 (1974) 136–143
3 *Holmes, M.J., P.J. O'Morchoe, C.C.C. O'Morchoe:* The role of renal lymph in hydronephrosis. Invest. Urol. 15 (1977) 215–219
4 *Kaplan, A., M. Friedman, H.E. Kruger:* Observations concerning the origin of renal lymph. Am. J. Physiol. 138 (1942) 553–556

5 *Nordquist, R.E., R.D. Bell, R.J. Sinclair, M.J. Keyl:* The distribution and ultrastructural morphology of lymphatic vessels in the canine renal cortex. Lymphology 6 (1973) 13–19
6 *O'Morchoe, C.C.C., K.H. Albertine:* The renal cortical lymphatic system in dogs with unimpeded lymph and urine flow. Anat. Rec. 198 (1980) 427–438
7 *Bell, R.D., M.J. Keyl, W.L. Parry:* Experimental study of lymph formation in the canine kidney. Invest. Urol. 8 (1970) 356–352
8 *Bell, R.D., R.J. Sinclair, W.L. Parry:* Influence of renal fluid dynamics on renal lymph flow, pressure and composition. Lymphology 3 (1974) 143–148
9 *Freie, J.T., R.D. Bell:* Renal fluid dynamic changes produced by arterial infusion of acetylcholine. Proc. Soc. Exp. Biol. Med. 138 (1971) 547–549
10 *Hinshaw, L.B.:* Mechanism of renal autoregulation – role of tissue pressure and description of a multifactor hypothesis. Circ. Res. (Suppl. 1) 15 (1964) 120–129
11 *Smith, H.W., N. Finkelstein, L. Aliminosa, B. Crawford, M. Graber:* The renal clearance of substituted hippuric acids and other aromatic acids in dog and man. J. Clin. Invest. 24 (1945) 388–404
12 *Bell, R.D.:* Cortical and medullary canine renal lymph formation during acetylcholine induced renal vasodilation. Lymphology 4 (1971) 74–78
13 *Staub, N.C.:* Pulmonary edema due to increased microvascular permeability to fluid and protein. Circ. Res. 43 (1978) 113–151
14 *Albertine, K.H., C.C.C. O'Morchoe:* An ultrastructural study of the transport pathways across arcuate, interlobar, hilar and capsular lymphatics in the dog kidney. Microvasc. Res. 21 (1981) 251–361
15 *Friedman, J.J.:* Microcirculation. In E.E. Selkurt (Ed.), *Physiology*. Little, Brown and Company Boston 1976
16 *Parker, J.C., H.J. Falgout, R.E. Parker, D.N. Granger, A.E. Taylor:* The effect of fluid volume loading on exclusion of interstitial albumin and lymph flow in the dog lung. Circ. Res. 45 (1979) 440–450
17 *Navar, P.D., L.G. Navar:* Relationship between colloid osmotic pressure and plasma protein concentration in the dog. Am. J. Physiol. 233 (1977) H295–H298

Richard D. Bell, Ph.D., Associate Professor, Department of Physiology, Chicago College of Osteopathic Medicine, 1122 E. 53rd Street, Chicago, Illinois 60615