THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE EFFECTS OF HISTAMINE AND PROSTAGLANDIN E_2 ON RENAL FLUID DYNAMICS, RENAL LYMPH FLOW AND COMPOSITION IN THE DOG

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

RICHARD JAMES SINCLAIR

Oklahoma City, Oklahoma

1973

THE EFFECTS OF HISTAMINE AND PROSTAGLANDIN E_2 ON RENAL FLUID DYNAMICS, RENAL LYMPH FLOW AND COMPOSITION IN THE DOG

APPROVED BY

DISSERTATION COMMITTEE

ACKNOWLEDGMENTS

It is with sincere appreciation that I acknowledge the encouragement and guidance of Dr. M. Jack Keyl throughout my graduate career. His example of independence and hard work greatly aided the completion of this research.

I am indebted to Dr. Richard D. Bell, VA Hospital, for unselfishly providing the laboratory and specialized equipment this work required in addition to serving on my protocol and dissertation reading committees.

Appreciation is extended to Dr. Joanne I. Moore, Department of Pharmacology and Dr. Lerner B. Hinshaw, VA Hospital, for serving on the author's protocol and dissertation reading committees and Dr. Rex D. Stith for serving on the author's dissertation reading committee. Dr. A. Kurt Weiss has shown a personal interest in my progress during the entire period of my graduate studies and for this I am sincerely grateful.

I wish to thank Dr. Jiro Nakano, Department of Pharmacology, for his advice and generosity, Dr. John Pike of the Upjohn Company, Kalamazoo, Michigan, for supplying the needed prostaglandin, and Ms. Linda L. Beverage for her expert technical assistance.

Special appreciation is extended to Dr. D. J. Dierschke, the University of Wisconsin, for encouraging the author to become a scientist.

Finally, I wish to thank my wife, Susan, for typing this manuscript.

This research was supported by NIH Grants-in-Aid HL-09879, 12832 and VA Hospital, Oklahoma City. The author was supported by NIH Training Grant HL-05859.

To George Arthur Clapper

(1915 - 1972)

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CHAPTER I

INTRODUCTION

The Lymphatic System

Lymphatic vessels are very difficult to see unless carefully injected with some colored material to make them visible. As early as 300 B.C. Herophilus, Erisistrates, Galen and other early scientists observed small vessels containing colorless or milky white fluids but did not understand the function of these vessels (3).

The Renaissance brought the work of Gasparo Aselli (1581-1626) who, in 1622, observed white lacteals spread in the mesentery of a recently well-fed dog and on pricking one with a scalpel was astounded to see a white, milky fluid gush out. Further experiments revealed that only fed animals exhibited this phenomenon. Pecquet, in 1651, observed the mesenteric lymphatics in man (an executed criminal) and traced the thoracic duct and cysterna chyli in the dog. The Swedish anatomist Rudbeck (1630-1708) and the Danish anatomist Bartholin (1616-1680) found and described lymphatics in several areas of the body. Bartholin first observed lymphatics leaving the liver, contrary to the concept that chyle flowed into the liver to aid in the formation of blood. In 1653 he called

these vessels "Lymphatics" (3). The word lymph is derived from a Latin word for water, lympha, hence a colorless fluid.

The eighteenth century brought extensive study of lymphatics by William Hunter, William Cruickshank and William Hewson. The concept that lymphatic vessels absorb in all parts of the body, not just in the small intestine, was developed by these investigators. In 1862, von Recklinghausen demonstrated that lymphatics were lined with endothelial cells, as were blood vessels (3).

The end of the nineteenth century brought advances, by such great physiologists as Carl Ludwig and E. H. Starling, in the understanding of capillary exchange and the physical forces involved in this exchange. Ludwig maintained that lymph was derived as a filtrate from blood, dependent on blood pressure. Heidenhain, however, felt that lymph was secreted by the endothelium of the lymph vessels. Starling's classical concept that capillary exchange is a balance of forces between the hydrostatic pressure of the blood and the colloid osmotic pressure of the blood (1) gave strength to the filtration theory of Ludwig. Further, the realization that minute blood vessels "leak" small amounts of protein which are then picked up by the lymphatic system and delivered back to the general circulation served as the basis for our present understanding of lymphatic function (2, 3, 4).

In addition to the role of lymphatics in the return of interstitial protein to the blood, lymphatic transport of lipids in the gut is well documented (201). The lymphatic system also serves to prevent the entry of bacteria and other foreign material to the general circulation by means of lymph nodes which, in effect, filter the lymph. Circulating in the lymph node are phagocytic lymphocytes which ingest and

destroy the foreign object. Metastatic tumor cells also travel via the lymphatic network and often lodge in lymph nodes. Lymph node biopsy is probably one of the most important methods of cancer diagnosis. Lymphatic study has grown steadily in the last few decades such that at least two comprehensive volumes (2, 3) and an entire journal[1] in its sixth volume are devoted to the subject.

Renal Lymph

Distribution. Ludwig and Savarykin (5) had observed, in 1863, lymphatic vessels swell as a result of restricting the outflow of blood and urine from the kidney. These investigators stated that parenchymal lymphatics were connected to both hilar and capsular lymphatic trunks although no data were included in this publication. In 1944, however, Pierce (6) carefully studied the distribution of renal lymphatics in the dog, rabbit and guinea pig primarily by the stab-injection of India ink into the renal parenchyma. The stab-injection method involves injection of a colored substance directly into the tissue. Lymphatics are then assumed to take up the substance and can then be identified. Many other structures may also fill with the colored substance, however, such as blood vessels and tubules. Also, lymphatics distal to the injection site may be the only ones visualized because the lymph flows away from the site. Retrograde injection of ink may eliminate some of these artifacts and also fill the smallest lymphatics since the filling pressure can overcome lymph pressure. Pierce was able to demonstrate lymphatics surrounding and accompanying interlobular, arcuate and interlobar blood

[1] Lymphology, official organ of the International Society of Lymphology. Georg-Thieme Verlag - Stuttgart, publishers.

vessels connecting with efferent capsular trunks. No convincing evidence was found concerning glomerular, peri-glomerular or medullary lymphatics. Rawson, however, presented evidence in 1949 of medullary lymphatics in a post-mortem human kidney permeated with carcinoma (7). No more positive morphological evidence exists for medullary lymphatics, only a great deal of negative evidence.

In later work, Bell, et al (8) injected India ink in a retrograde manner into capsular lymphatics of dog and horse and hilar lymphatics in a calf. The hilar lymphatics of the dog contain many valves which make a retrograde injection impossible. The injected capsular lymphatics were seen to follow the course of interlobular blood vessels, extend to the cortico-medullary border and surround the arcuate blood vessels in a plexus of small lymphatics. Small lymphatic vessels were often seen associated with glomeruli in both dog and horse. Injection of hilar lymphatics in the calf resulted in the same cortico-medullary distribution observed in the dog. In none of the animals were any medullary lymphatics seen. Electron microscopic observations in 1973 by Nordquist, et al (9) revealed lymphatic capillaries in association with all major cortical structures in the dog kidney.

Renal lymph composition. Sugarman, et al (10) collected capsular and hilar renal lymph from the dog. Analysis revealed a protein concentration that varied with lymph flow, the highest protein concentration with the lower flow. There was no difference between hilar and capsular lymph with respect to protein concentration. Renal lymph was collected in volumes of 0.5 to 1.0 ml in 20-40 min periods regardless of flow rate. No attempt was made to prevent dehydration of these small samples during the collection period. In contrast, other investigators

have shown that when renal lymph flow is dramatically increased by means of renal arterial infusion of acetylcholine or increased renal venous pressure, lymph protein concentration does not change (11, 12, 13). Lymph protein composition could be altered, however, by occluding the renal artery for five minutes and then allowing blood flow to return. The first post-occlusion lymph sample contains significantly more total protein than the pre-occlusion samples. Flow does not change from control following the occlusion and presumably the additional protein was left behind in the renal interstitium during the period of no blood flow by a reversal of pressure gradients. Upon reinstatement of blood flow the first lymph sample to be collected is protein-rich. Permeabilities were not altered as evidenced by no change in lymph flow (14). Renal lymph has been shown by many others to contain about half the protein of arterial plasma while all the different plasma proteins are represented (4, 15, 16, 17). The percentage of albumins in lymph is greater than in plasma (15) but it is not surprising considering the difference in size of the albumin and globulin protein molecules. Capsular renal lymph is somewhat higher in protein content in the unanesthetized dog (18).

Renal lymph is generally similar to arterial plasma in electrolyte composition although some investigators have reported that sodium and chloride were higher in lymph (16) and that urea, inulin and PAH were lower in renal lymph (15) than arterial plasma.

Lymph flow. Renal lymph flow rates have been reported to be quite variable, ranging from 2.2 to 14 ml/hr under steady-state hemodynamic conditions (4, 10, 11, 12, 13, 15, 16, 17, 18). Flow rates will vary depending on the number and size of anastomotic lymph channels and, of course, the hemodynamic state of the animal. The size of the lymph

catheter is very important, also. Flow will decrease when a relatively constant pressure acts against a high resistance, such as the very small I.D. tubing used in lymph flow measurements.

Guyton, et al (19) and Courtice (20) feel that the tissue pressure, or pressure of the interstitial fluid, squeezes the terminal lymphatic capillaries, moving the fluid inside into the next larger lymphatic vessel. The terminal lymphatic then recoils from the squeeze of the tissue pressure with the overlapping endothelial cells of the lymphatic opening to admit more fluid. These endothelial cells are anchored by filaments which prevent them from collapsing inward. Any motion in the tissue, such as muscular contraction, respiration or arterial pulsation add to the movement along the lymphatics. Although the large hilar and capsular lymph channels of the kidney have not been studied for intrinsic contractility and rhythmicity, other lymph trunks of comparable size have been tested. Lymphatics of the bat's wing (21) and mesenteric lymphatics (22) have been studied and found to have spontaneous rhythmic contractions. This activity is shown also in the most recent work (23) on bovine mesenteric lymphatics. Two to three cm. sections of lymphatic were isolated in a muscle bath and found to have spontaneous, rhythmic contractions of 2/min. Norepinephrine increased the frequency of contraction and could be blocked by phentolamine, suggesting alpha-adrenergic receptors. Likewise, isoproterenol induced slowing of contraction and could be blocked by propranolol, suggesting the presence of beta-adrenergic receptors.

The rate of production of renal lymph is probably the primary variable in determining lymph flow rate and the hemodynamic state of the

kidney is responsible for the production of lymph.

Renal Fluid Dynamics

Anatomy of the Renal Vascular Bed

The renal artery divides upon entering the hilus into several branches which divide again to pass to the cortico-medullary border as the interlobar arteries. These branches then bend around parallel to the renal capsule as arcuate arteries which give off small branches into the cortex toward the capsule. These small branches are interlobular arteries which each supply fifty to one hundred fifty glomeruli. A short afferent arteriole leaves the interlobular artery to supply a single glomerulus, although occasionally two or more may be supplied. Certain other small arterial supplies, such as arteriae rectae verae, which supply some vasa recta, vasa varorum, and those to the renal capsule, bypass the glomerulus entirely.

The afferent arteriole divides into a number of small, intertwining branches within Bowman's capsule becoming the glomerular capillary tuft. These branches leave the capsule near their point of entry and recombine to form the efferent arteriole which divides once more into peritubular capillaries. Efferent arterioles from glomeruli adjacent to the cortico-medullary border, or juxtamedullary glomeruli, turn toward the medulla following the loops of Henle and divide into several vasa recta. At variable distances into the medulla these vessels turn back toward the cortex to join in finally to the arcuate venous system.

The peritubular capillaries and vasa recta eventually form larger venous channels and drain into interlobular, arcuate and interlobar veins, eventually joining as the renal vein and emptying directly

into the inferior vena cava. A network of veins, the stellate veins, lie under the renal capsule in some species and collect outer cortical blood. These veins junction at discrete points from which the blood enters an interlobular vein. Valves are found in the renal, interlobar and arcuate veins (24, 25).

The vascular bed of the kidney is supplied with sympathetic nerve fibers derived from T_4 to L_4 and distributed thru the celiac and renal plexuses (24). The principal function appears to be production of acute, transitory reduction of renal blood flow by vasoconstriction as a general response to stress. Section or block is followed by no detectable change in blood flow or glomerular filtration rate, but stimulation is followed by large decreases in flow (25). Adrenergic fibers innervate vessels as far as afferent arterioles in the cortex and outer medullary vasa recta (26). The same distribution of acetylcholinesterase-containing fibers has been found (27) and suggests the possibility of sympathetic cholinergic innervation.

Renal Blood Flow

Renal blood flow through both kidneys of man or dog is about twenty-five percent of the cardiac output at rest (24, 25, 28). Perfusion of each kidney in the anesthetized or conscious dog is three to four ml/min per gram of kidney which exceeds the perfusion of almost any other organ in the body. Flow is distributed primarily in the cortex with only about seven percent in the medulla (25).

The first direct renal blood flow measurement was by Landergren and Tigerstedt in 1893 by using a stromuhr, developed by Carl Ludwig, inserted in the renal artery (24), Burton-Opitz and Lucas (1908) modified

the stromuhr so it would fit the renal vein. Mason, et al (1936) introduced a device into the vena cava that occluded above and below the renal vein so that renal venous effluent could be collected (28). Collection of venous effluent remained the standard method of direct renal blood flow measurement until the advent of electronic, continuous reading devices such as the electromagnetic flowmeter.

Using a thermostromuhr that he developed, Rein (29) reported in 1931 that canine renal blood flow remained constant during alterations in systemic arterial pressure. This property of the kidney and other organs is referred to as autoregulation (24). Over a range of about ninety to two hundred mm Hg arterial pressure, renal blood flow does not change. Renal vascular resistance increases with increased pressure while flow remains constant. This phenomenon occurs in the dog, cat, rat and rabbit and is assumed to exist in man (25). No satisfactory explanation of the mechanism of this phenomenon is yet available.

Intrarenal Pressures

Swann and his associates (30, 31) developed a method in 1950 by which they could study intrarenal pressures. They inserted a twenty gauge needle, connected to a manometer, into the kidney parenchyma and determined the pressure at the tip of the needle. This pressure averaged about 25 mm Hg in the dog.

Swann postulated that the luminal pressures within the kidney, including tubules, lymphatics, capillaries and venules should be the same or should exceed 25 mm Hg to remain patent. To test his hypothesis he inserted a catheter in a retrograde manner into the renal vein of a dog so that the tip lay in an arcuate vein. The pressure found here was the

same as the concurrently recorded intrarenal pressure (24 mm Hg). The two pressures followed each other with a correlation coefficient of 0.85 when the kidney was subjected to glucose diuresis, epinephrine infusion and elevated ureteral pressure. Withdrawal of the catheter revealed a pressure drop to 7 mm Hg just distal to the confluence of the arcuates (32). Koester, et al (33) earlier had found sinusoidal cushions located at the confluence of the arcuate and interlobar veins which, in effect, dam up the venous effluent keeping the peritubular capillary pressure elevated to that approximating tubular pressure. The kidney is then kept inflated by the hydrostatic pressure imparted by the heart and by the inelastic capsule which surrounds it. Other investigators using essentially the same techniques have confirmed the intrarenal needle pressure to be in the range reported by Swann (34, 35). Intrarenal needle pressure in the rat has been reported as being 10mm Hg (34).

Micropuncture measurements by Gottshalk (36) in the rat revealed that proximal tubular pressure averaged about 13mm Hg and about 7mm Hg in distal tubules. He also found peritubular capillary pressure to be essentially the same as proximal tubular pressure suggesting that the interstitial pressure in the rat kidney is essentially the same, so that transmural pressures would be small.

The relation of tissue pressure, lymph flow and deep venous pressure subsequently interested investigators studying the mechanisms by which the kidney autoregulates.

Although Swann discarded the hypothesis that tissue pressure controlled renal autoregulation (37), Hinshaw tested the idea using the needle pressure technique (38, 39, 40, 41, 42, 43) and comparing tissue pressure to deep venous pressure (41, 42, 43). The result seemed to lend

credence to the hypothesis that tissue pressure, or extravascular pressure, had an important role in autoregulation. Hinshaw developed a valuable tool in perfecting the techniques of intrarenal venous pressure (IRVP) for determiniation of segmental vascular resistances in the kidney and further established the role of deep venous pressures of the kidney in renal function (44, 45).

Studies involving deep venous pressures and their relation to other aspects of renal function have been recently reported. Martino and Earley (46) used deep intrarenal venous pressure as an index of renal capillary pressure to see if physically induced changes in sodium reabsorption are mediated by forces across the capillary. Gazitua, et al (47) reported only small changes in deep venous pressures and hilar lymphatic pressure with increasing renal blood flow. Bell, et al (13) suggested that the kidney is maintained in a state of elevated venous presure because of a constriction in the intrarenal veins (33). This elevated venous pressure may determine lymph formation and interstitial fluid pressure in the kidney. They found that lymph pressure and tissue pressure increase immediately in response to elevations in IRVP in the same way that IRVP increases when renal vein pressure is increased (12).

Renal function and hemodynamics can be altered dramatically by many pharmacological agents in order to study various renal physiological parameters. While a large number of these agents have been studied, especially acetylcholine (14, 48, 49, 50, 51) in regard to renal function and renal fluid dynamics, little is known concerning two other important naturally occurring vasoactive agents, histamine and prostaglandin.

Histamine

History

Dale and Barger began, in 1904, to study the pharmacology of ergot, a fungus which attacks rye. This particular agent is very toxic if consumed but was useful as an abortifacient (52).

Windhaus and Vogt (53) synthesized histamine in 1907 but were unaware that it was one of the potent pharmacological agents in ergot. Barger and Dale (54) and Kutscher (55) identified and isolated this agent as the same material - Histamine - prepared by Ackermann (56) from the amino acid histadine by bacterial decarboxylation.

Early investigation on the effects of histamine by Dale and Laidlaw (57, 58), Burn and Dale (59) and Dale and Richards (60) revealed a strong depressor effect on vascular smooth muscle while Popielski (61) and Keeton, et al (62) demonstrated a powerful stimulation of gastric secretion.

To date, the physiological significance of histamine is unknown. The most recent theory is that it is but an evolutionary vestige and holds physiological function only in invertebrates (63).

Chemistry and Occurrence

The empirical formula $C_5H_9N_3$ represents 4 (or 5) - (2-aminoethyl) imidazole, or histamine. It is not a complex compound for one which occurs naturally, consisting of an imidazole ring with one aminoethyl group at the fourth carbon position (64).

Histamine has been found in blood, blood vessels, bone marrow, ailementary canal, kidney, liver, lung, heart, muscle, brain and nervous

system, reproductive organs, skin and urine of all invertebrate and vertebrate species studied (63, 65). Histamine is stored primarily within mast cells or circulating basophils in granules, bound to heparin. Much histamine is also found in non-mast cell sites such as epidermis, central nervous system and gastro-intestinal mucosa (66).

Biosynthesis and Metabolism

A specific histidine decarboxylase requiring a cofactor pyridoxal-5-phosphate produces histamine *in vivo*. This enzyme has been located in mast cells and basophils (63, 66, 67) in granules bound to heparin.

Metabolism of histamine occurs primarily through imidazole ring N-methylation by histamine-N-methyltransferase to methylhistamine which is acted on by monoamine oxidase (MAO) to yield methyl imidazole acetic acid which is excreted in the urine. Histamine also undergoes oxidative deamination by diamine oxidases (histaminase) to imidazole acetic acid which conjugates with ribose and is excreted in the urine (64, 66).

Pharmacology

The release of histamine from its bound state can be accomplished by tissue damage from trauma of mechanical, thermal or radiant energies (66) and is implicated in the "triple response" of Lewis (68). Antigen-antibody reactions and chemical agents such as enzymes, surface active agents, venoms and histamine-releasing drugs (e.g. compound 48/80) cause histamine release by widely varying mechanisms.

A large number of agents are available which antagonize the effects of histamine. These "anti-histamines" act by occupying histamine receptor sites on the effector cells. It is entirely a competative

inhibition in that there is no chemical reaction between agonist and antagonist. Some of the most commonly used agents are diphenhydramine HCl (Benadryl), dimenhydrinate (Dramamine), and chlorpheniramine maleate (Chlor-trimeton) (15).

Histamine stimulates isolated guinea pig uteri and cat stomach muscle in a manner resembling adrenergic stimulation (69) but also stimulates uterine and bronchiolar smooth muscle independent of innervation (66).

Popielski (61), Keeton, et al (62) and Ivy and Bachrach (70) found that subcutaneous administration of histamine resulted in a strong stimulation of gastric secretion in dogs, rats, cats and humans. Histamine was shown to be present in the intestinal mucosa by Barger and Dale (71) but was differentiated from the antral hormone gastrin by Komorov in 1938 (72).

Khalson, et al (73) found that the injection of gastrin in fasted rats resulted in gastric nucosal release of histamine with an increase in mucosal histidine decarboxylase. He suggested a model in which mucosal histamine is released by gastrin to stimulate secretion by the parietal cell. Johnson and Aures (74) and Johnson (75) conclude that histamine is not the intermediate and that gastrin stimulates the parietal cell directly. The controversy continues.

Histamine occurs in autonomic nerves and may be functionally connected with adrenergic neurons (76). Burn and Dale (59) observed in 1926 that infusion of histamine in the cat released catecholamines from the adrenal medulla, later shown to be a direct action on the chromaffin cell (66).

Histamine administered intravenously in the cat or dog (57, 58) lowers the blood pressure while that of an ether-anesthetized rabbit is raised. Large intravenous doses result in the "Histamine Shock" of Dale and Laidlaw (77) with peripheral vasodilation and increased vascular permeability, resulting in reduced venous return and cardiac output. The hematocrit increases while lymph flow also increases.

The isolated mammalian heart responds to histamine with increased rate and force (78). These responses are like those to catecholamines or sympathetic stimulation and may result from the histamine-induced release of catecholamines (78, 79).

Dilation results from topical application of histamine to arterioles, capillaries and venules of rat mesoappendix and mesocecum (80, 81), infusion of histamine into dog forelimb arteries (82, 83), dog hindlimb arteries (84, 85), cat hindlimb arteries (86), rabbit ear arterioles (87) and arterioles in cat and dog skin and skeletal muscle (88). However, constriction of some of the same vascular beds also has been reported (77).

Haddy demonstrated, in the dog foreleg, slow rates of intra-arterial histamine infusion (2 - 14 μg/min) increased blood flow with an increased venous pressure due to arteriolar dilation while higher infusion rates (greater than 14 μg/min) dilated arterioles and actively constricted veins. The venous constriction results from direct histamine action and indirectly through adrenal discharge (83). Diana, et al (84) studied the isolated dog hindlimb at constant blood flow or constant pressure and determined that intra-arterial histamine produces both arteriolar and venous dilation. Diana and Kaiser (85) in 1970 demonstrated that any dose of intra-arterial histamine (1 - 50 μg/kg/min) produced general venodilation in the isolated dog hindlimb. Precapillary and postcapillary

resistance decreased while capillary pressure dropped. The authors concluded that histamine does not increase capillary pressure as a result of venous constriction and increased postcapillary resistance as Haddy (83) had suggested as the mechanism of histamine edema. Lewis and Winsey (86) infused histamine, and other vasoactive substances, intra-arterially in the in situ cat hindlimb and observed that histamine increased blood flow and femoral lymph flow in a dose-related manner. No consistent lymph flow response to increased venous pressure alone was found but when venous pressure was elevated at a time when vascular permeability was high, as a result of histamine infusion, there was a consistent increase in lymph flow.

Spector (89) in his review on substances affecting capillary permeability defined "increased capillary permeability" as ". . . . an alteration in the capillary wall leading to an accelerated rate of passage of plasma proteins into tissues." Histamine was one of the main topics of his review and discussed in the greatest detail. Majno, et al (90), however, found no argument with the fundamental concept that increased blood vessel permeability exists as a result of agents such as histamine, but questioned the site of the "leak".

Based on electron microscopic evidence (91) that histamine and serotonin (5-HT) cause leaks to appear between endothelial cells while the basement membrane remains intact, they decided to follow suspended particles in the presence of such an endothelial leak. Colloidal carbon was injected intravenously into rats followed by a subcutaneous injection of histamine (or 5-HT) into the scrotum. The cremaster muscle from the scrotum was then excised, fixed and cleared for examination. The leaking vessels were found to be on the venous side of the circulation, especially

in venules 20-30 micra in diameter. The smallest vessels, which were probably capillaries, did not develop endothelial leaks. Gabbiani, et al (92) tested these results by injecting histamine (and other agents) intra-arterially in rats following intravenous carbon black. Histamine injected intra-arterially caused vascular leakage, again, predominantly from the venules. In contrast to the ealier work of Majno, et al (90), the agent reached the arteries before the venules and from the endothelial, or luminal side. The authors concluded that histamine-type mediators have a selective effect on the venules of susceptible organs.

A recent study was conducted, using Majno's technique, on the effects of histamine and histamine-type mediators deleivered to the rat kidney by a variety of methods, including intra-arterially (93). Leakage of colloidal carbon could not be detected in the renal parenchyma, while it appeared with the usual endothelial contraction in the perirenal tissue. The authors suggested some peculiarity of the renal parenchyma was responsible

The Kidney

There is a paucity of information in the literature concerning the effects of histamine on renal fuction. Dale and Laidlaw in 1910 (57) observed a systemic depressor response, decreased urine flow and decreased kidney volume in cats given 500 µg histamine I.V. Morimoto, as early as 1928, injected histamine into the renal arteries of cats and dogs and found constriction even at minimal doses (94).

Bjering (95) gave subcutaneous histamine to patients and noted decreased arterial pressure with a fall in urea and creatinine clearances. Reubi and Futcher (96) observed a decreased renal plasma flow with no

change in GFR in normotensive and hypertensive patients with subcutaneous doses of 0.3 - 0.5 mg. Blackmore, et al (97) studied the effects of I.V. histamine on unanesthetized dogs at 2.5 μg/kg/min for two hours. GFR remained constant but increased after cessation of the infusion. Renal blood flow increased and remained elevated until one hour after cessation of the infusion while the filtration fraction and arterial pressure declined. Urine flow decreased during the infusion, leading the authors to suggest an antidiuretic role for histamine. More recent work concerning the effects of histamine on renal hemodynamics and renal function is not available.

Prostaglandin

History

Prostaglandins are described as vasodepressor, smooth muscle stimulating, acidic lipids (98). Structurally they are twenty-carbon carboxylic acids synthesized from polyunsaturated fatty acids in vivo by the incorporation of three oxygen atoms on a five membered ring. The precursor of prostaglandin, arachidonic acid, is a fatty acid which is the principal component of the cell membrane, the probable site of prostaglandin formation (99).

Kurzrok and Lieb (100) observed that, in attempting artificial insemination on human subjects, semen injected into the uterine cavity was expelled. The same volume of Ringers solution was retained. Following up their observations in the laboratory they discovered that isolated strips of human uterus would contract or relax in the presence of fresh human semen. Spermatazoa were not required for this action.

The investigators concluded that some intrinsic property of both uteri and semen was responsible.

Later in the 1930's Goldblatt in England (101) and von Euler in Sweden (102) observed independently that human seminal plasma or the extract of sheep seminal vesicles had strong stimulating activity on uterine smooth muscle and would sharply reduce blood pressure when injected into an animal. Von Euler found this activity in the seminal fluid of monkey, sheep and goat concentrated in the lipid soluble acid fraction. He named it Prostaglandin (103, 104).

Chemistry and Occurrence

Bergström and Sjöval found that the biological activity of prostaglandin was due to lipid soluble unsaturated hydroxy acids and then isolated the first two crystalline prostaglandins (105, 106). Bergström, et al then demonstrated the presence of prostaglandin E (PGE) in ram seminal plasma (107). Ultramicroanalysis and mass spectrometry of prostaglandin yielded the empirical formulae $C_{20}H_{34}O_5$ (PGE) and $C_{20}H_{36}O_5$ (PGF) (108). The structure of PGE_1 is (-) 11α, 15 (5) dihydroxy -9-oxo-13-trans-prostanoic acid. Prostaglandins have twenty carbon atoms with a prostanoic acid skeleton. PGE's contain 11α -hydroxy and 9-keto groups on a five-membered ring. They are really dehydrated by weak alkali to a 10:11 unsaturated ketone (Prostaglandin A - PGA) and can rearrange to PGB, a doubly conjugated ketone. Prostaglandin F (PGF) is analogous to PGE but with the 9-keto group reduced to a hydroxyl.

All primary prostaglandins contain a 13:14 trans double bond. E_1 and F_1 prostaglandins contain one double bond while E_2 and F_2 have two double bonds (5:6 cis). Similarly, E_3 and F_3 have another double bond

at the 17:18 cis position (109).

The primary prostaglandins (E, A, F) occur naturally in lung (110), thymus (111), brain and spinal cord (112), kidney (113, 114, 115), iris (116), umbilical cord (117), human decidua (118), fat and adrenals (119), ovaries (120), spleen (121), intestine (122) and nerves (123).

Biosynthesis and Metabolism

Bergström, et al (124) and van Dorp, et al (125) found that homo-α-linoleic acid, arachidonic acid and all-cis-eicosa 5, 8, 11, 14, 17 pentaenoic acid are transformed in high yields to PGE_1, PGE_2 and PGE_3, respectively, when incubated with whole homogenates of sheep vesicular glands. These reactions also occur in bovine seminal vesicles (126), iris (127), intestinal mucosa (128) and brain (129). A microsomal enzyme, prostaglandin synthetase, effects the biosynthesis (109, 130).

Small amounts of tritiated PGE_1 administered intravenously in rats results in fifty percent recovery in urine and ten percent in feces, via biliary excretion, within forty hours. High concentrations of isotope are found in liver and kidney with smaller concentrations in lungs, pituitary, adrenals, ovaries, uterus and heart. Very low concentrations are found in brain, muscle, adipose tissue and thymus (131). During one circulation through vascular beds PGE_1 and PGE_2 are rapidly removed from the blood. More than ninety percent of intra-arterially infused PGE_1 or PGE_2 disappeared in one circulation through the lungs of cats, rabbits and dogs. Less was removed through the cat liver and about fifty percent through the cat hindquarters (121). The prostaglandin degrading enzymes 15-hydroxy prostaglandin dehydrogenase and prostaglandin Δ^{13}-reductase have recently been localized in swine lung, spleen and kidney (132).

Pharmacology

Prostaglandins cause contraction or relaxation of smooth muscle which may be modified by changing the ionic composition of the medium, various hormones, blocking agents and interaction with other agonists.

In 1967 Strong and Bohr (133) reported the effects of PGE_1, E_2, A_1 and $F_{1\alpha}$ on isolated vascular smooth muscle from rabbits, rats and dogs. Although coronary and aortic strips contract in the presence of these prostaglandins, small artery muscle relaxes even when pretreated with catecholamine or potassium chloride. The response to prostaglandin is calcium dependent and not blocked by alpha or beta adrenergic blocking agents and antimuscarinics (133, 134), antihistamines (133) or serotonin (134).

While pharmacological blocking agents are not useful in studying prostaglandins, the search for a specific prostaglandin antagonist continues. PGE_1 contraction of isolated gerbil intestinal muscle is antagonized by PG analogue 7-oxa-13-prostynoic acid in a dose related manner. The antagonistic action is not entirely specific, however, since the effects of acetylcholine and histamine are also reduced (135). Dose-related inhibition of PGE_1 and PGE_2 on guinea pig ileum, consistent with competitive inhibition, results from the use of SC-19220, a dibenzoxazepine hydrazide derivative. This drug has no effect on bradykinin, acetylcholine (136) or histamine (137) induced contractions. Polyphloretin phosphate acts as a competitive antagonist of PGE_2 and $PGF_{2\alpha}$ on the isolated gerbil colon (138) but does not inhibit sertonin, angiotensin, bradykinin or acetylcholine induced contractions (139).

Evidence for the interaction of prostaglandins and the sympathetic nervous system is rapidly accumulating. Coceani suggested in 1966

that the increased sensitivity of the isolated stomach fundus of the rat to PGE_1 after treatment with procaine, bretylium, dichloroisoproterenol, dibenamine or hexamethonium results from blockage of intrinsic nerves, especially sympathetic fibers (140). Clegg applied E and F prostaglandins to various isolated smooth muscles in subthreshold doses and observed diminished effects of the sympathomimetic agents epinephrine, norepinephrine, phenylephrine and isoproterenol (141). PGE_1 and E_2 can also induce persistent sensitivity increases in the guinea pig uterus to other agonists such as vasopressin, oxytocin, histamine, electrical field stimulation and $PGF_{2\alpha}$. Enhancement of sensitivity is not seen after pretreatment with $PGF_{1\alpha}$, $PGF_{2\alpha}$ ricinoleic acid, sodium phosphatidate, hydroperoxide-containing linoleic acid or oxytocin. The degree of potentiation is parallel to the PGE_1 concentration up to about 1.2 ng/ml. Higher concentrations result in no greater enhancement.

While low calcium or high magnesium decrease the contractile response to PGE_1, they do not affect PGE_1 enhancement of vasopressin or electrically induced contractions (142). PGE_1 has also been reported to enhance the response to acetylcholine in the rat stomach fundus (140). Angiotensin and serotonin induced contractions, but not those caused by norepinephrine are enhanced by PGE_1 in the rabbit aortic strip (143).

Noting the decrease in norepinephrine release from the isolated, electrically stimulated rabbit heart when perfused with PGE_2, Hedqvist, et al suggested that PGE_2 modulates sympathetic nerve transmission (144). PGE_1 and PGE_2 also inhibit the effects of sympathetic stimulation on isolated guinea pig atria, contraction of isolated guinea pig vas deferens (145), and outflow of norepinephrine from the cat spleen (146). The data

imply that prostaglandin may inhibit the release of norepinephrine from nerve endings. Hedqvist, et al (144) and Hedqvist (146) feel that PGE_2 may be a physiological modulator of sympathetic function operating in a negative feedback system.

Isolated rat and guinea pig uteri contract when treated with E and F_α prostaglandins (147) while PGE, A and F decrease the tonus, frequency and amplitude of the spontaneous contractions of isolated human uterine strips (148). Ovariectomized rats pretreated with estrogen yield uteri which are less sensitive to PGE_1 and $PGF_{2\alpha}$ but with increased sensitivity to oxytocin. Progesterone pretreatment was without effect (149).

Intravenous prostaglandins E_1, E_2 and A_1 strongly inhibit gastric secretion in unanesthetized dogs (150) while PGE_1 relaxes in vitro rat duodenum (143), circular muscle of intestine and colon (151) and reduced antral and intestinal motility in dogs (152). I.V. PGE_1 results in abdominal cramping in humans (153). PGE, however, lowers blood pressure in rats but does not stimulate the intestines (154). $PGF_{2\alpha}$ contracts circular and longitudinal intestinal smooth muscle (155) and increases intestinal motility (152).

Boyarsky, et al (156) reported in 1966 that I.V. PGE_1 slows or stops peristalsis of the dog ureter in vivo or applied in vitro in a dose dependent manner while $PGF_{2\alpha}$ (157) increases peristalsis in vivo.

Prostaglandins, arachidonic acid and prostaglandin enzymes are found in sheep (158), rabbit (159) and pig iris (127) while I.V. depressor doses of PGE_1 elevate intra-ocular pressure in rabbits but not in cats and monkeys after intra-ocular injection (160). Ramwell and Shaw have found prostaglandins and prostaglandin-like substances in brain and released spontaneously from cat cerebral cortex. They suggest that

prostaglandins may have central nervous system actions (119).

Prostaglandin E_1 lowers the arterial pressure in the dog (161), cat (162), rabbit (163), rat (164) and chick (165). The single I.V. dose which is minimally effective is between 1 - 10 µg/kg (109) and the depressor action persists after atropine (147), antihistamines and ganglionic blocking agents, pretreatment with reserpine (153) and beta-blocking agents (166). The I.V. infusion of dimethylphenylpiperazinium bromide (DMPP) or electrical stimulation of the central ends of the cut vagus raised blood pressure in dogs receiving a continuous infusion of PGE_1. The pressor reponses of vasopressin and angiotensin are diminished by PGE_1 (162). Heart rate in the dog increases along with the depressor response but it is prevented by beta-blockade (166).

Prostaglandins E and A are powerful direct acting coronary vasodilators in the intact dog (167) and E and $F_{1\alpha}$ in the dog heart-lung preparation (168). $PGF_{2\alpha}$ is without positive inotropic effects while PGE_1 and A_1 increase myocardial force. All increase cardiac output (167). Katori and Takeda find $PGF_{2\alpha}$ to have some "feeble" inotropic action in the isolated dog heart (168).

Nakano and McCurdy (169) and Nakano (167) studied the cardiovascular effects of PGE_1, A_1 and $F_{2\alpha}$ on the intact dog finding that prostaglandins exert their effects primarily by a potent action on the peripheral arterial bed: PGE and A dilating, $PGF_{2\alpha}$ constricting.

Kidney

Observations that bilaterally nephrectomized dogs develop hypertension (experimental renoprival hypertension) (170) and that renal homotransplants in dogs (171) and parabiotic attachment in rats (172)

reduced the hypertension led to the concept that the kidney possesses an endocrine, anti-hypertensive function. This blood pressure lowering factor was thought to be in the kidney cortex and be related to adenosine nucleotides (173, 174) but was shown to reside in the renal medulla by Muirhead, et al (175). Extracts of canine renal medulla lowered the blood pressure of dogs with renoprival hypertension and appeared to prevent the development of hypertension during extended experiments.

In 1965, Lee, et al (176) isolated a depressor substance from rabbit renal medulla he called "medullin". The substance closely resembled PGE_1 in chemical and physiological properties but did not stimulate non-vascular smooth muscle. Two other isolates from rabbit medulla were identified as PGE and PGF. Thin-layer chromatographic, spectroscopic and mass spectral analysis of rabbit renal medulla identified PGE_2, PGA_2 and $PGF_{2\alpha}$ (177). In 1970 Crowshaw, et al (178) found PGE_2, A_2 and $F_{2\alpha}$ in dog medulla suggesting that these agents might be important new renal hormones.

Prostaglandin E_2 and the other renomedullary prostaglandins are localized in the inner medulla (179, 180), can be synthesized there (180, 181) and are readily metabolized by the kidney (182). Enzymes for the degradation of prostaglandins (prostaglandin dehydrogenase and Δ^{13}-reductase) are found in the kidney, especially the cortex (132).

Studies by McGiff, et al on the release of renomedullary prostaglandins has shown prostaglandin activity released from dog kidneys in response to renal ischemia (183) and during the intra-arterial infusion of either norepinephrine or angiotensin II (184). Fujimoto and Lockett (185) reported in 1970 that an intravenous infusion of norepinephrine, but not angiotensin, released a prostaglandin-like material into the

renal lymph of cats. They suggested that the diuretic action of norepinephrine may be mediated by prostaglandin release. Norepinephrine infused into the renal artery decreases blood flow and urine formation but a recovery occurs in one to three minutes. This compensatory reaction occurs with the appearance of PGE in renal venous blood (198). Renal nerve stimulation results in initially the same effects but there is neither recovery nor prostaglandin release. Prostaglandin concentration in renal venous blood during release averaged 0.9 ng/ml while the threshold arterial concentration needed for vasodilation and diuresis is only 0.1 ng/ml (184). Norepinephrine infused into the renal artery may first constrict the cortical vessels, forcing the drug into the medulla where prostaglandins are formed. Norepinephrine released from renal nerves would be limited to the cortex. However, Dunham and Zimmerman found release of prostaglandin-like material when the nerves to an isolated dog kidney were stimulated as well as when norepinephrine was infused directly (186).

Prostaglandin E_1 or E_2 infused directly into the renal arteries of dogs increases renal blood flow (169, 187-192) and decreases renal resistance with no decrease in either man or pulsatile arterial pressure. Dosages reported vary with the investigator from a bolus injection of 0.1 μg/kg (169) to prolonged renal arterial infusions of 0.01 to 2.0 μg/min (187).

The functional response was first reported by Johnston, et al (187) when glomerular filtration rate did not change, renal plasma flow increased, PAH extraction decreased, urine osmolality dropped and the excretion of sodium increased. The authors suggested that prostaglandin's role in the kidney is intrarenal control of resistance and salt and/or

water balance. There were significant increases in free water clearance despite large exogenous doses of vasopressin, suggesting a direct tubular effect in addition to hemodynamic changes at postglomerular sites, indicated by stable GFR's. Vander (188) infused either PGE_1 or PGE_2 intra-arterially, confirming the work of Johnston, et al and additionally demonstrating that there was no difference in the effects of either PGE_1 or PGE_2 on the kidney. He also suggested that PGE had a direct tubular effect since as little as 20 ng/min would initiate natriuresis. Many other investigators have infused E prostaglandins into dogs (192, 189-192, 194), rats (195) and humans (196) reporting natriuresis, kaliuresis, diuresis, decreased urine osmolality, increased osmolar clearance, and increased free water clearance. Additionally they found increased urea, calcium, magnesium and phosphorous excretion, increased PAH transport and clearance with a variable change in GFR and increased plasma renin activity. Blood flow shifts in both medulla and cortex and dilation of interlobular and arcuate arteries have also been reported. Medullary sodium and urea concentrations were decreased in dogs (189) and micropuncture studies in rats (195) revealed proximal sodium reabsorption was unchanged, suggesting an effect at a more distal site on the nephron.

Prostaglandin A_1 infused intravenously into hypertensive patients (197) increased effective renal plasma flow, GFR, urinary flow, urinary sodium and potassium excretion.

Hypothesis

There is a paucity of information in the literature concerning the effects of histamine on renal function and fluid dynamics. Conversely, there is a wealth of information available on the effects of

prostaglandins on renal function but little concerning renal fluid dynamics as it relates to renal lymph flow and production. The object of this research was a comparison of the effects of these two vasoactive agents on renal fluid dynamics and lymph flow.

There are certainly parallels between the history of the study of histamine and prostaglandin on both the general physiology and the kidney. It is clear from the above discussion that both agents have been implicated - at different times - in intrarenal control of renal function. Neither agent, however, has any clear physiological function.

Lee (199) in his recent review on renal prostaglandin has suggested that this ubiquitous lipid serves as the "natriuretic hormone" which many renal physiologists believe exists. There is other strong evidence that prostaglandin serves as the controlling factor in renal autoregulation (200).

For the above reasons this research was designed to answer the following questions:

1) Does histamine or PGE_2 alter lymph protein concentration, thereby suggesting altered vascular permeability to protein?

2) How does histamine or PGE_2 affect renal lymph flow and production in relation to changes in blood flow and intrarenal vascular pressures?

3) Can the lymph flow responses to histamine or PGE_2 be based on changes in blood flow alone?

4) Although these two agents have widely varying potencies in this preparation, do they differ in the vascular structure affected?

5) Finally, can a comprehensive statement be made on the

possible role of these naturally occuring vasoactive agents in the physiological control of renal fluid dynamics?

CHAPTER II

METHODS AND MATERIALS

Experimental Animals

Forty-eight mongrel dogs of both sexes (18 - 26 kg), obtained from professional dealers, were utilized for this research. Twenty-two animals were considered successful preparations while six animals served to test drug dosage levels.

Each animal was conditioned by treatment for removal of endo- and ectoparasites and fed a daily ration of commercial dog food, both canned and dry. Water was allowed ad lib whereas food was withheld twenty-four hours prior to an experiment.

Anesthesia

Anesthesia was induced by I.V. injection of sodium pentobarbital (Nembutal Sodium-Abbott) 30 mg/kg (202) of body weight. A surgical plane of anesthesia was maintained by additional doses of 60-120 mg as required. The depth of anesthesia was determined by the absence of reflex to nervous stimuli applied to a hind limb or the absence of the eyelid reflex.

Surgical Preparation

The anesthetized animals were secured on a surgical table in a lateral recumbent position by heavy cord tied loosely to front and hind

limbs. An incision was made over the right femoral artery and vein and these vessels were then carefully isolated. In some experiments the left femoral artery was isolated in the same manner. The femoral vein was catheterized with polyethylene tubing of appropriate size to facilitate the administration of additional anesthetic. The right (and/or left) femoral arteries were catheterized with PE 205 polyethylene tubing (Clay Adams - I.D. = 1.57mm) to facilitate the collection of arterial blood samples and the monitoring of central arterial pressure. In some experiments the right femoral artery was used to introduce an infusion catheter, so the left femoral artery was used for pressure monitoring. All catheters were filled with heparinized saline (Heparin Sodium - HW&D 10mg/100cc saline).

The left kidney was exposed via a flank incision caudal to the rib cage and extending from the level of the lateral spinous processes ventrally to the rectus abdominus muscle. The abdominal muscles and peritoneum were retracted. The kidney was carefully dissected free from the dorsal wall and ventrally retracted to expose the hilus.

The renal artery was carefully cleared of connective tissue to enable the proper placement of the blood flow probe. Care was taken not to injure the usual plexus of nerves and lymphatics surrounding the renal artery.

A curved 20 gauge needle was inserted into the renal artery for the infusion of saline and the experimental drug. The needle was connected to the infusion pump by a length of PE 90 polyethylene tubing (Clay Adams - I.D. = 0.86mm). In some experiments an angiographic catheter (Cordis disposable angiographic catheter, model 523-841) was

introduced into the right femoral artery and the tip moved in a retrograde manner until it lay in the left renal artery. The tip could be visualized and gently palpated in the artery. This catheter was firmly secured at the femoral incision. Infusions were accomplished by the use of a Harvard syringe pump (model 940) calibrated to deliver 0.2 cc/min.

Renal Blood Flow

An electromagnetic flow meter (Micron RC 1000) was used to continuously monitor renal blood flow (RBF). A flow probe (Micron, model MC and Clark Associates, model CA) of an appropriate size to be slightly constrictive was carefully applied to the renal artery. A probe 10-15 percent smaller than the artery results in a better "fit" with less variability of contact between vessel wall and electrode, hence less noise signal and artifact (203).

Each probe was calibrated by means of measurement of direct femoral arterial bleed-out with graduated cylinder and stop watch. Care was taken to clean the flow probe electrodes with an abrasive cleanser before and after each use.

Experimental Drugs

Histamine

Histamine Phosphate, U.S.P. (Histamine Disphosphate Fisher Scientific Co. Lot #7025) was dissolved in normal saline immediately prior to use. Any solution remaining after the termination of the experiment was discarded.

Prostaglandin

Prostaglandin E_2 (PGE_2) was supplied by Dr. John Pike of the Upjohn Company as crystalline PGE_2 (Lot # U-12062). This was prepared in ethanol and sodium carbonate solution (per instructions from Upjohn) to a strength of 1 mg/ml.

This method of preparation, unfortunately, is not satisfactory. The aqueous solution rapidly loses potency, probably through dehydration and conversion to PGB. Brummer (204) has reported that the storage life of PGE_2 in ethanol at -15° C is at least one year while aqueous solutions remain potent for only one week to one month. Subsequent samples of PGE_2 (Lot # U-12062) were dissolved in 95 percent ethanol and kept frozen. Sufficient PGE_2 solution in saline was prepared for each experiment and any remaining solution was discarded.

Control infusions. Control and recovery infusions in the histamine experiments consisted of normal saline. Control and recovery infusion in the PGE_2 experiments consisted of a prostaglandin vehicle with the same concentration of ethanol and sodium carbonate as in the experimental solution. No control solution caused any significant change in any parameter studied.

Drug dosages. The dosage of histamine phosphate or PGE_2 which results in a maximal blood flow response without systemic depressor responses were determined by trail in six dogs. Maximal renal hemodynamic effects were desired because of their action on lymph flow and pressure. Alteration in systemic pressure, however, would introduce the variable of changing sympathetic tone to the kidney along with general sympathoadrenal discharge. The dosage of histamine phosphate selected was 3 μg/kg/min and of PGE_2 0.4 μg/kg/min, both delivered into the renal

artery at 0.2 cc/min.

Experimental Procedures

This project involved two separate kinds of procedure. In one preparation, renal blood flow (RBF), systemic arterial pressure (AP), urine flow and osmolality, renal hilar lymph flow and lymph and plasma total protein concentration were determined. In the second preparation renal blood flow, systemic arterial pressure, renal vein pressure (RVP), renal small vein pressure (SVP), subcapsular pressure (SCP) and renal capsular lymph pressure (L_CP) were monitored.

Lymph Collection Experiments

Renal blood flow was determined as described above. Systemic arterial pressure was recorded by means of the femoral arterial catheter connected to a Statham P23Db strain-gauge transducer. Both mean RBF and mean AP were recorded continuously on a Sanborn model 7700 recorder.

The left ureter was isolated and catheterized with PE 90 tubing which was advanced to the level of the renal pelvis. Slight traction was put on the ureter to prevent kinking of the tubing or blockage by the ureteral wall. Timed urine collections were made continuously in graduated centrifuge tubes.

One of the several hilar lymphatic trunks running parallel to the renal artery was carefully isolated and ligated with 4-0 silk. PE 10 polyethylene tubing (Clay Adams - O.D. = .16mm) with a beveled tip was inserted with a small forceps through the lymphatic wall into the lumen in one motion. After observing free lymph flow, the tubing was secured in the vessel with 4-0 silk.

At this point the animal was systemically heparinized (2 mg/kg) to prevent clotting of the lymph and blood. This dose was usually sufficient for the duration of the experiment.

The experiemental period itself was one hour with collection of lymph and blood at five-minute intervals. Three five-minute control periods were followed by six five-minute experimental periods during which the experimental agent was infused. Three five-minute recovery periods followed. Five minutes was arbitrarily chosen as the minimum time a lymph sample could be collected and delivered to the reaction vessel and the next collection tube readied.

Five cc of arterial blood was collected from the arterial catheter while lymph was collected in 20 µl disposable capillary pipettes (Yankee micropet) which were connected directly to the lymph catheter by a short piece of larger tubing. These pipettes are calibrated to be within $\pm\frac{1}{2}$ percent of their stated volume of saline. At the end of the experiment the animal was euthanized with a lethal dose of Nembutal. The left kidney was then removed, decapsulated weighed.

Renal Fluid Dynamics

RBF and AP were determined as described above. The left gonadal vein was isolated and catheterized with Clay Adams PE 90 tubing and the catheter tip advanced into the renal vein for measurement of renal venous pressure (RVP). The RVP catheter was connected to a Hewlett Packard model 1280 strain-gauge transducer.

Small vein pressure (SVP) in the kidney was measured by a catheter (Clay Adams PE 90) threaded up the renal venous system via a 20-gauge needle hole in the renal vein. The criteria of Hinshaw (45) for

proper catheter placement were followed:

1) a pressure increase when the catheter tip was advanced into veins deep in the parenchyma,

2) a rapid return in pressure following catheter flushing,

3) free withdrawal of blood through the catheter.

This catheter was connected to a Hewlett Packard model 1280 strain-gauge transducer.

Renal subcapsular pressure (SCP) was used as a measure of renal tissue pressure by utilizing a miniature wafer-type pressure transducer (Sensotec model M-7BW). The microtransducer (6.35 mm diameter and 0.5 mm thick) was introduced via a 1 cc slit in the renal capsule and gently advanced about 3-4 cm under the capsule.

A capsular lymphatic vessel was used for lymphatic pressure measurements. Freie and Bell (51) have shown that pressures recorded from capsular vessels more nearly approximate intrarenal pressures than do hilar pressures. Hilar lymphatic outflow circuits are arranged in a parallel manner so that increased pressure in one channel is relieved by the others.

The lymphatic was catheterized with PE 60 polyethylene tubing (Clay Adams O.D. = 1.2 mm) which has been drawn down at the tip to allow entry into the lumen. The catheter could be wedged into the vessel to prevent leakage and, in most cases, suturing was not required. The vessel could be tested for leaks by gentle stroking of the lymphatic toward the catheter tip and observing no decrease in lymphatic pressure. The lymphatic catheter was connected to a Hewlett-Packard model 1280 strain-gauge transducer and all pressures and flows were continuously monitored on a Sanborn 7700 recorder.

The experimental periods were as before with three 5 minute control periods, six 5 minute experimental periods followed by three 5 minute recovery periods. At the end of the experimental period the animal was terminated with a lethal dose of nembutal and the left kidney was removed, decapsulated and weighed.

Lymph Protein Analysis

Total protein concentrations in lymph and arterial plasma samples were determined by the biuret method (205). This method detects peptide bonds by reaction with copper sulfate in alkaline solution and is reliable up to about 11 gm percent protein. A commercial stable biuret reagent (Hycel, lot numbers 70915, A0329) was used with a commercial protein standard (Dade Reagents Lab-Trol lot number LT-33J). Standard curves were prepared to 7 gm percent.

Twenty μl samples of lymph and arterial plasma were transferred to test tubes with 1.5 cc biuret reagent kept on ice or in a refrigerator. When all samples had been collected, the tubes were transferred to a 37°C constant-temperature water bath for 30 minutes. Immediately following incubation the samples were read on a Coleman Jr. II spectrophotometer at 540 mu. Percent transmittance readings were compared to the standard curves and the results in gm protein/100 ml plasma expressed as lymph to arterial plasma concentration ratios for total protein, (L_H/P).

Urine Osmolality

Urine osmolality was determined by freezing point depression with an Advanced Instruments osmometer, model 3W. Standard solutions of 100 and 900 mOsm/kg H_2O were utilized and 1.5 cc of sample was required.

Each increase in osmolality of 1 mOsm/kg lowers the solution freezing point 0.001858°C. The model 3W osmometer supercools the solution several degrees below the freezing point at which time it vibrates it internally. Ice crystals begin to form with the release of heat of fusion until equilibrium (freezing point) is reached at the temperature read. The temperature at equilibrium is a function of the concentration of the sample. Accuracy is within 1 percent, based on standard solutions.

Vascular Resistances

Renal vascular resistances were calculated as follows:

$$\text{Total Renal Resistance} = \frac{\text{mean AP - RVP}}{\text{RBF}}$$

$$\text{Prevenous Resistance} = \frac{\text{mean AP - SVP}}{\text{RBF}}$$

$$\text{Venous Resistance} = \frac{\text{SVP - RVP}}{\text{RBF}}$$

Values were expressed as peripheral resistance units (mmHg/ml/min).

Statistical Analysis

Student's t-test for paired data (206) was used to determine probability values. A probability value of 0.05 or less was considered "statistically significant".

CHAPTER III

RESULTS

Renal Blood Flow

Histamine, when infused into the renal artery (3 μg/kg/min), increased RBF significantly from an average control value of 4.3 ml/min/gm kidney wt to a mean experimental flow of 6.0 ml/min/gm (Figure 1). RBF remained elevated during the entire experimental period (30 min) but rapidly dropped toward control when saline was substituted for histamine. Control and recovery periods were not significantly different.

The same relationship occurred when PGE_2 (0.4 μg/kg/min) was infused into the renal artery. Flow increased significantly from a mean control value of 4.4 ml/min/gm to an experimental average of 7.7 ml/min/gm. Upon termination of the prostaglandin infusion RBF returned to control values (Figure 2).

RBF was significantly higher as a result of PGE_2 infusion than with histamine while control flows were not significantly different between the two experimental groups, as shown in Figure 3.

Urine Flow and Osmolality

Histamine infusion increased urine flow from 0.43 ml/min to an average 0.97 ml/min which returned to control at the end of the drug infusion (Figure 4). Osmolality significantly decreased from an average

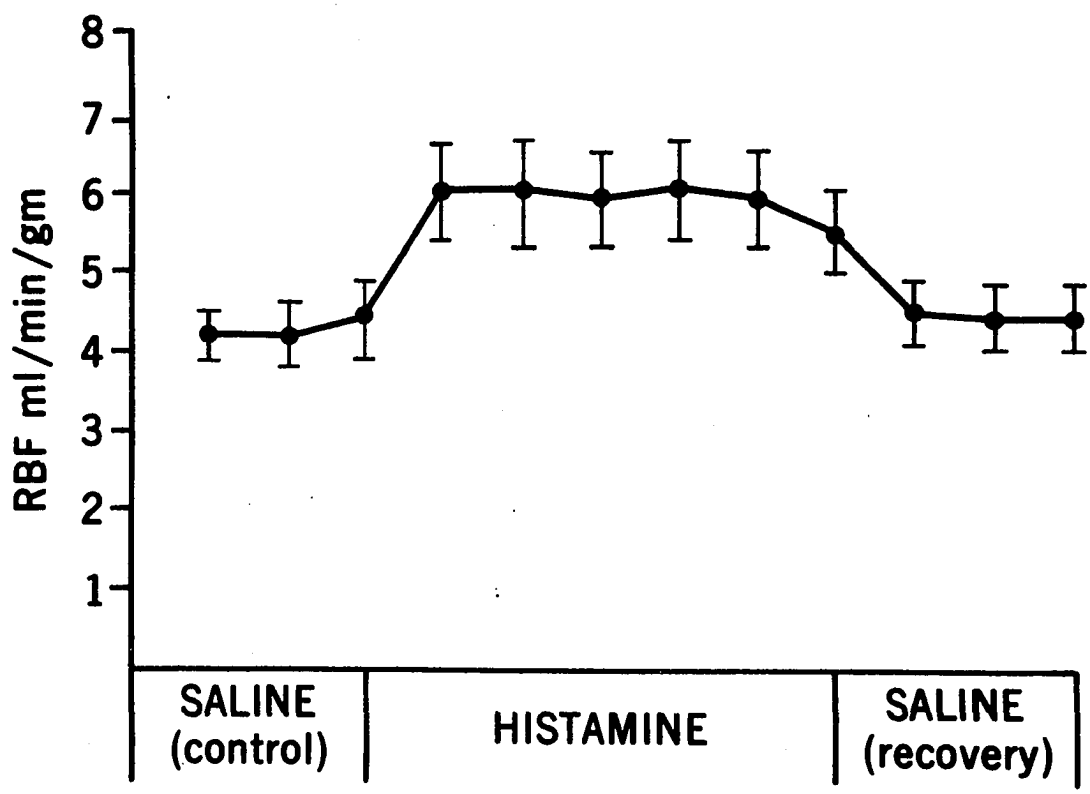

Figure 1. Renal blood flow (RBF) before, during and following renal arterial infusion of histamine with observations at five min intervals. Means and standard errors for ten animals shown.

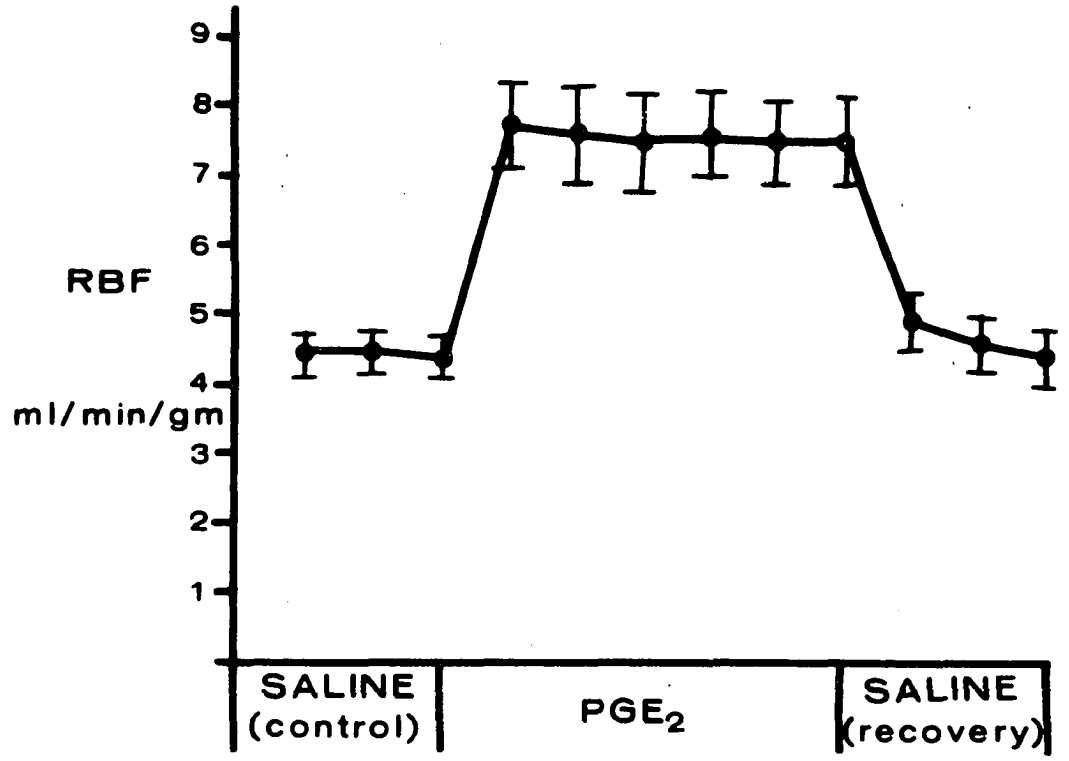

Figure 2. Renal blood flow (RBF) before, during and after renal arterial infusion of PGE_2 with observations at five min intervals. Means and standard errors for eleven animals shown.

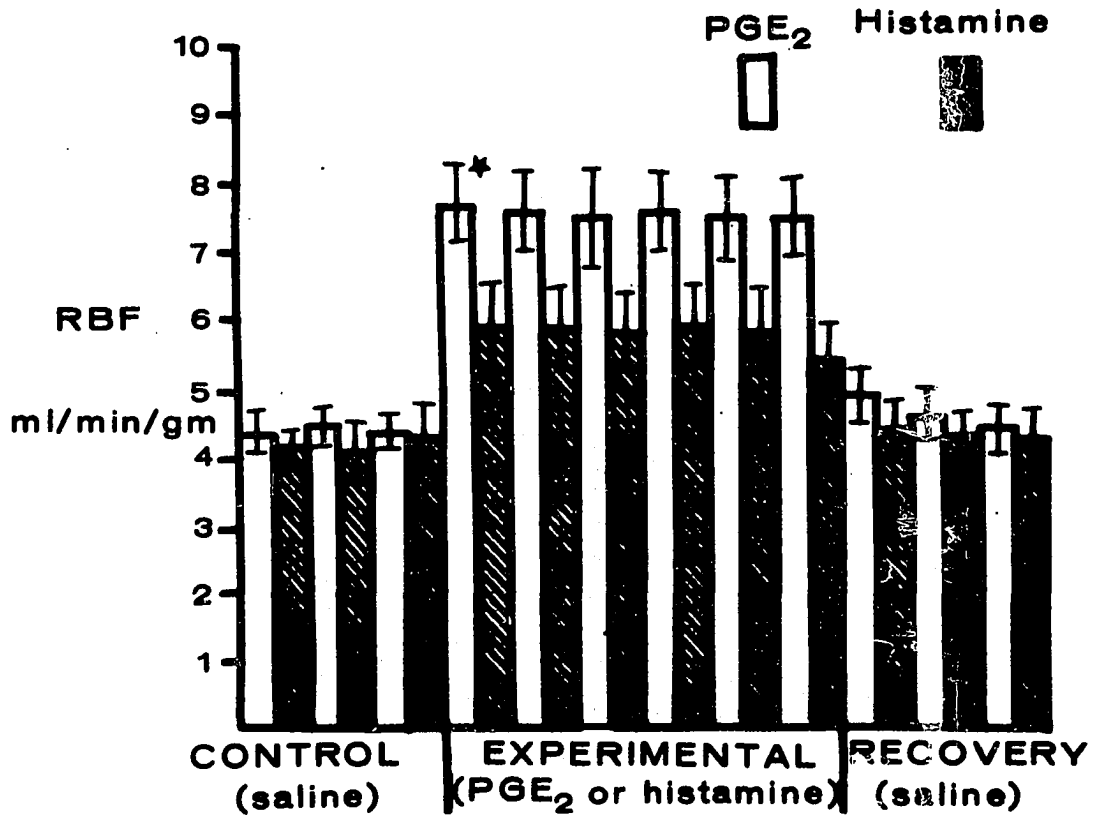

Figure 3. Comparison of renal blood flows (RBF) before, during and after renal arterial infusion of PGE_2 (n=11) or histamine (n=10). Each observation made at five min intervals. Means and standard errors shown. * $p < 0.01$ represents a significant difference between first PGE_2 experimental period and first histamine experimental period.

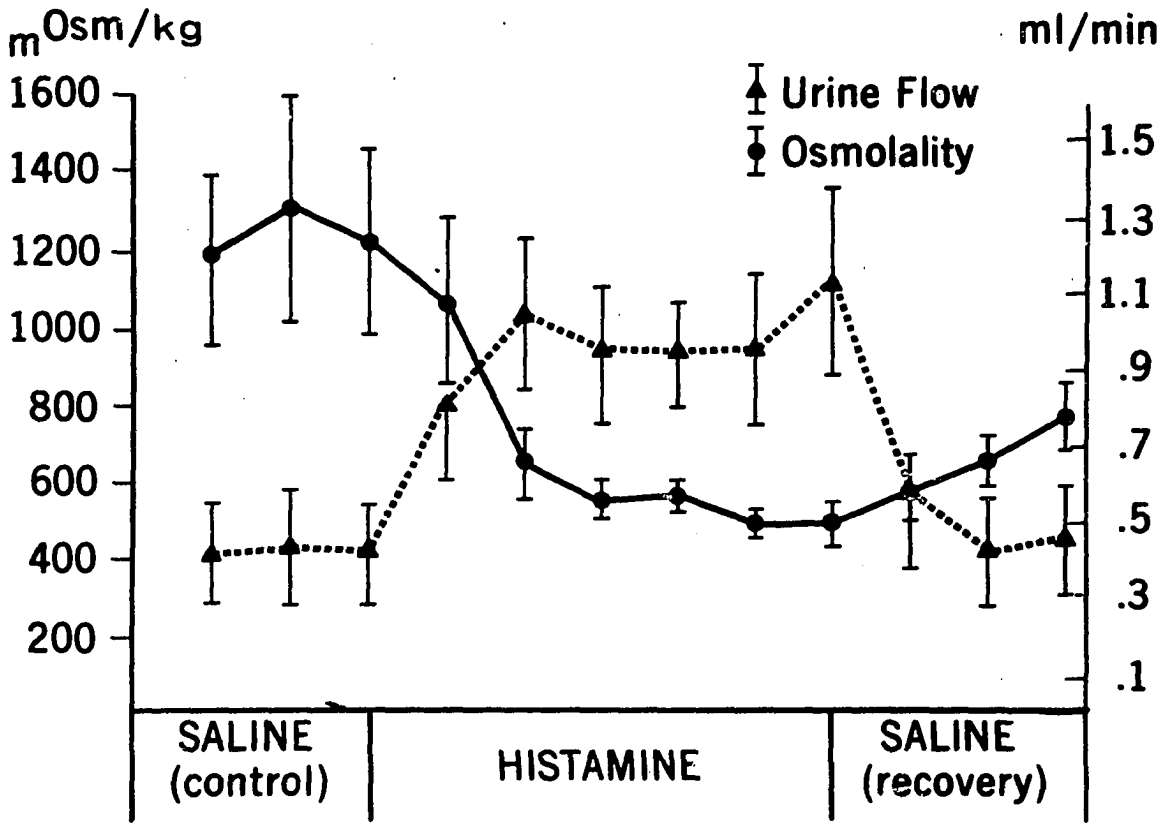

Figure 4. Urine flow and urine osmolality before, during and after renal arterial infusion of histamine. Each observation made at five min intervals. Means and standard errors for five animals shown.

1239 mOsm/kg to 644 mOsm/kg. Osmolality very gradually returned to control values during the recovery period.

PGE_2 increased urine flow from a mean control value of 0.24 ml/min to 1.10 ml/min. Urine osmolality declined from 1186 mOsm/kg to 510 mOsm/kg (Figure 5). Urine flow rapidly returned to control values upon cessation of the prostaglandin infusion while osmolality of the urine returned very slowly to control.

There was no significant difference between the two groups in either control or experimental periods, with respect to urine flow. Control urine flows in the PGE_2 dogs were uniformly, but not significantly, lower than in the histamine dogs while experimental urine flow values reached the same levels. Urine osmolalities decreased to about the same values and slowly returned to control in both groups, suggesting a common mechanism.

Renal Venous Pressure

Mean renal venous pressure (RVP) did not significantly change during the course of either the histamine (Figure 6) or the PGE_2 (Figure 8) experiments. Figure 7, however, illustrates the small vein pressure (SVP) change during histamine infusion. SVP increased significantly from an average control value of 23 mm Hg to an average experimental value of 32 mm Hg. PGE_2 infusion increased SVP from a mean control value of 19 mm Hg to an experimental value of 28 mm Hg (Figure 9). Control and experimental RVP and SVP values between the histamine and PGE_2 groups were not significantly different.

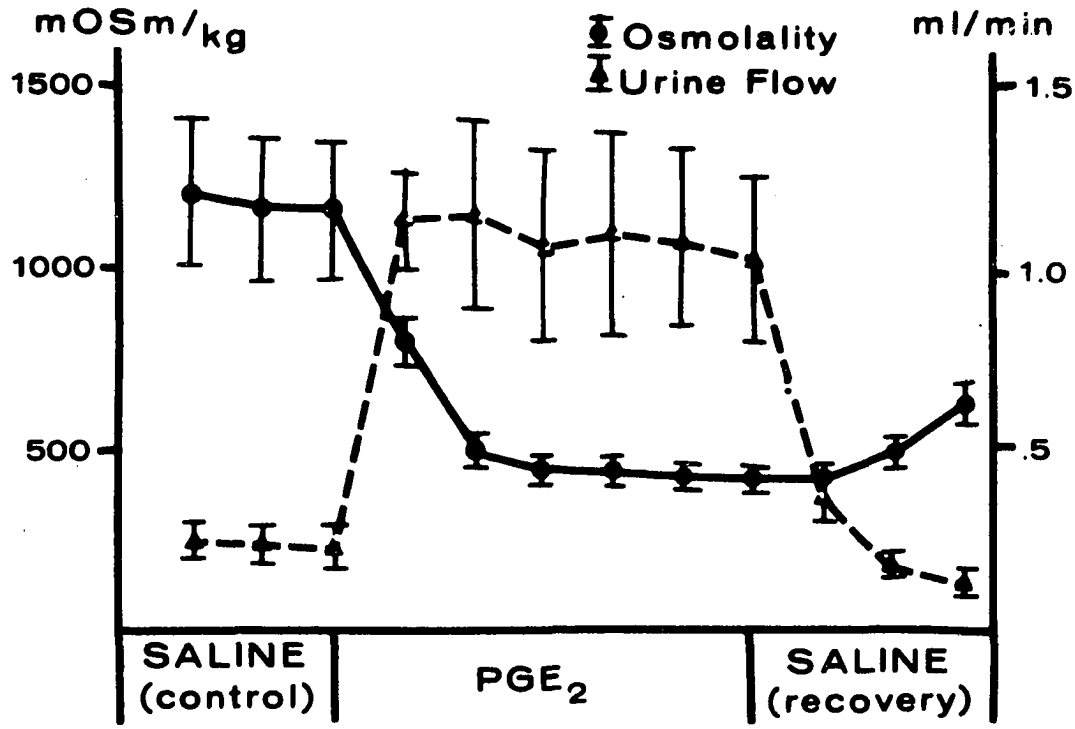

Figure 5. Urine flow and urine osmolality before, during and after renal arterial infusion of PGE_2. Means and standard errors shown for five animals. Each observation made at five minute intervals.

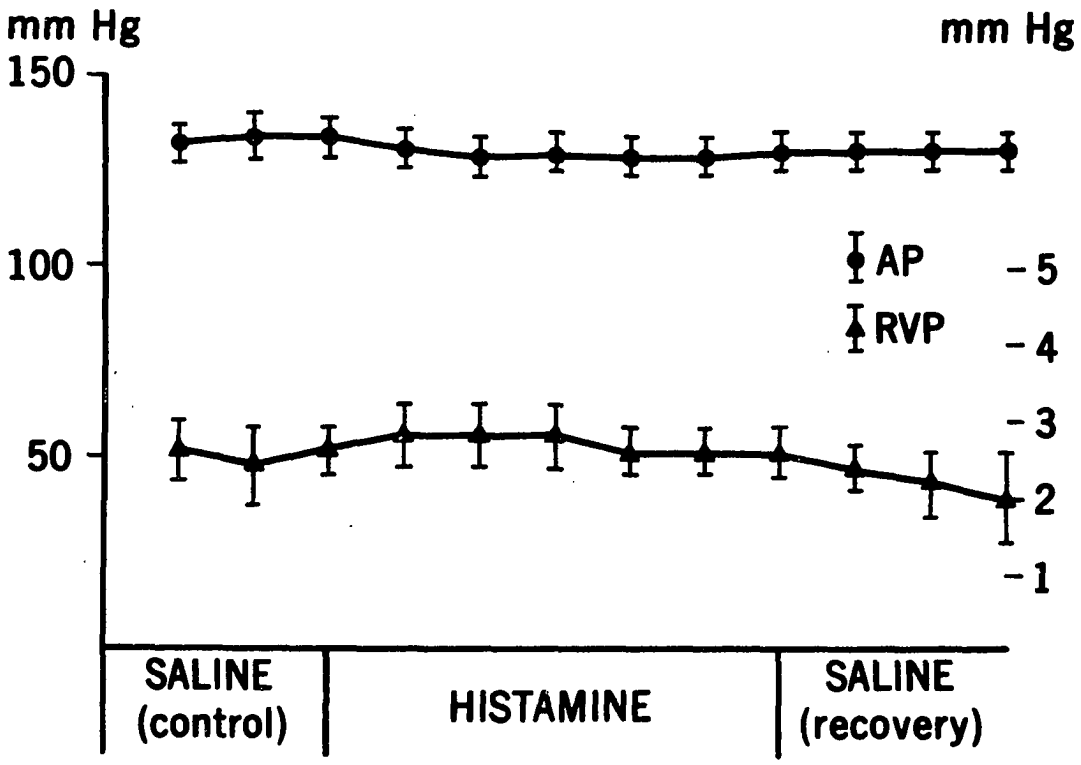

Figure 6. Mean arterial pressure (AP) and mean renal venous pressure (RVP) before, during and after renal arterial infusion of histamine. Each observation made at five min intervals. Means and standard errors for ten animals (AP) and five animals (RVP) shown.

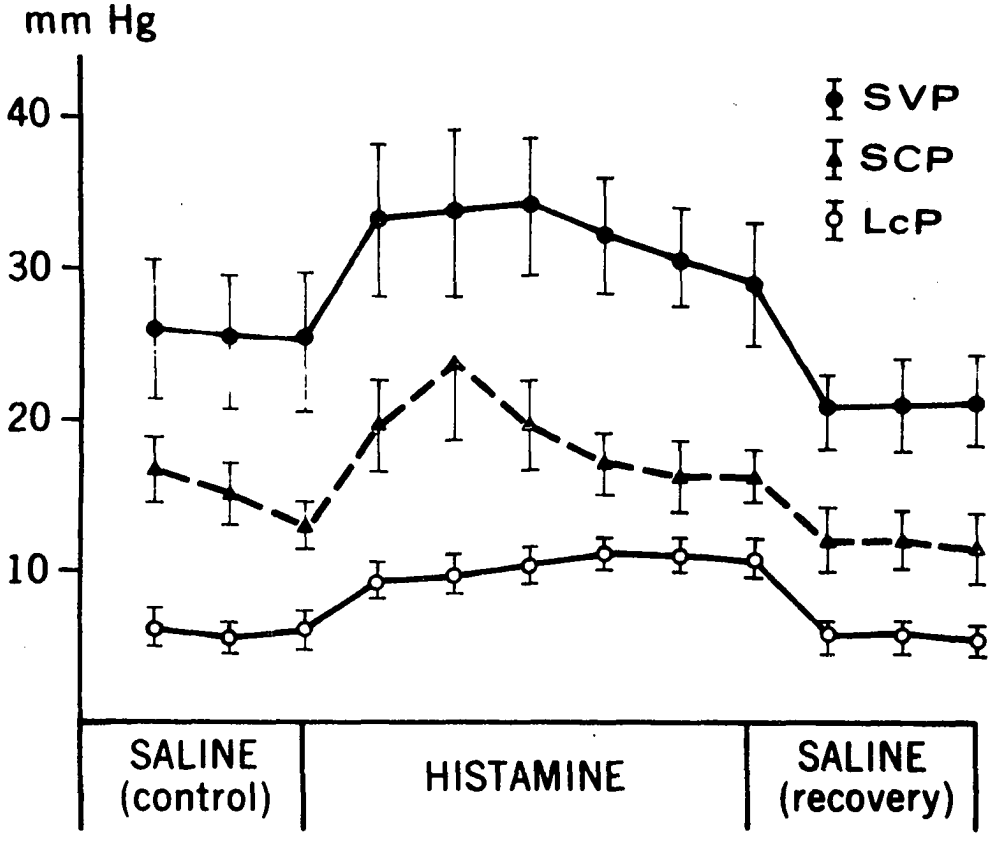

Figure 7. Renal small vein pressure (SVP), subcapsular pressure (SCP) and capsular lymph pressure (L_cP) before, during and following renal arterial infusion of histamine. Each observation made at five min intervals. Means and standard errors shown for five animals.

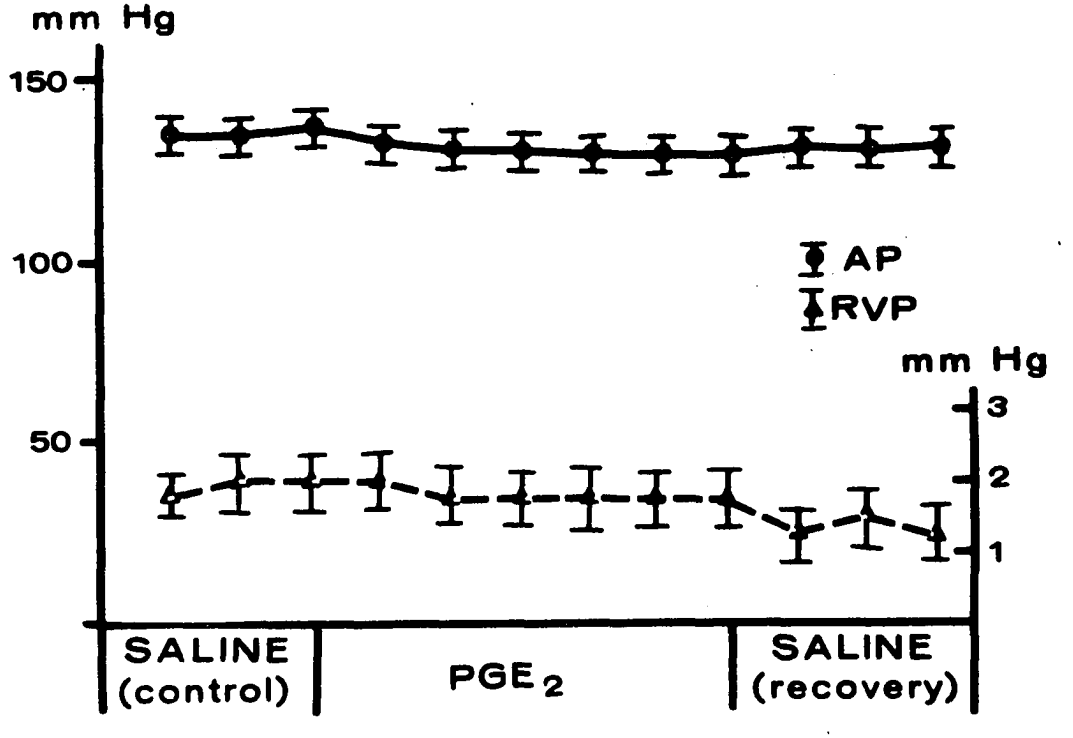

Figure 8. Mean arterial pressure (AP) and mean renal venous pressure (RVP) before, during and after renal arterial infusion of PGE_2. Each observation made at five min intervals. Means and standard errors for twelve animals (AP) and six animals (RVP) shown.

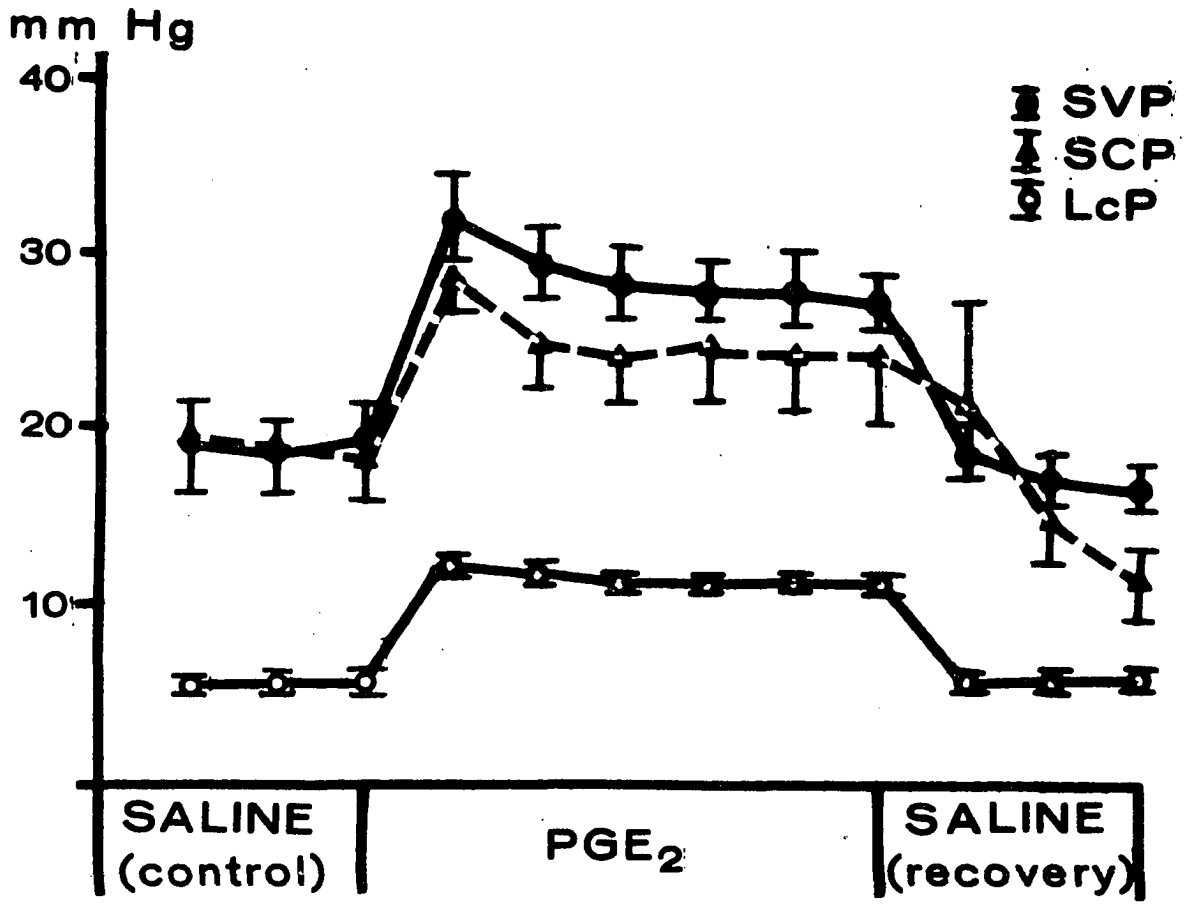

Figure 9. Renal small vein pressure (SVP), subcapsular pressure (SCP) and capsular lymph pressure (L_cP) before, during and after renal arterial infusion of PGE_2. Each observation made at five min intervals. Mean and standard errors for five animals shown.

Arterial Pressures

Mean arterial pressure did not change significantly with histamine (Figure 6) infusion but did change significantly during infusion of PGE_2 (Figure 8). This change, however, averaged less than 5 mm Hg which is the smallest division calibrated on the recorder record. This change was therefore considered within reading error. Arterial pressures between the two groups were not different.

Renal Vascular Resistance

Total renal resistance (R_t) significantly decreased with the infusion of histamine from an average control value of 0.60 peripheral resistance units (P.R.U.) to a mean experimental value of 0.42 P.R.U. (Figure 10). Similarly, PGE_2 decreased R_t significantly from 0.59 P.R.U. to an average 0.34 P.R.U. as seen in Figure 11.

Prevenous resistance (R_p) changed significantly from an average 0.48 P.R.U. to a mean experimental value of 0.34 P.R.U. during histamine infusion while PGE_2 resulted in a significant R_p change from 0.54 P.R.U. to an average 0.27 P.R.U. Control and experimental resistance values between the histamine and PGE_2 groups were not significantly different.

In contrast to R_t and R_p, renal venous resistance (R_v) did not change significantly with the infusion of either drug. The average R_v during the histamine experiments was 0.10 P.R.U. while R_v during the PGE_2 experiments averaged 0.07 P.R.U.

Subcapsular Pressure

Histamine infusion changed renal subcapsular pressure (SCP) from an average control value of 16 mm Hg to a mean experimental value

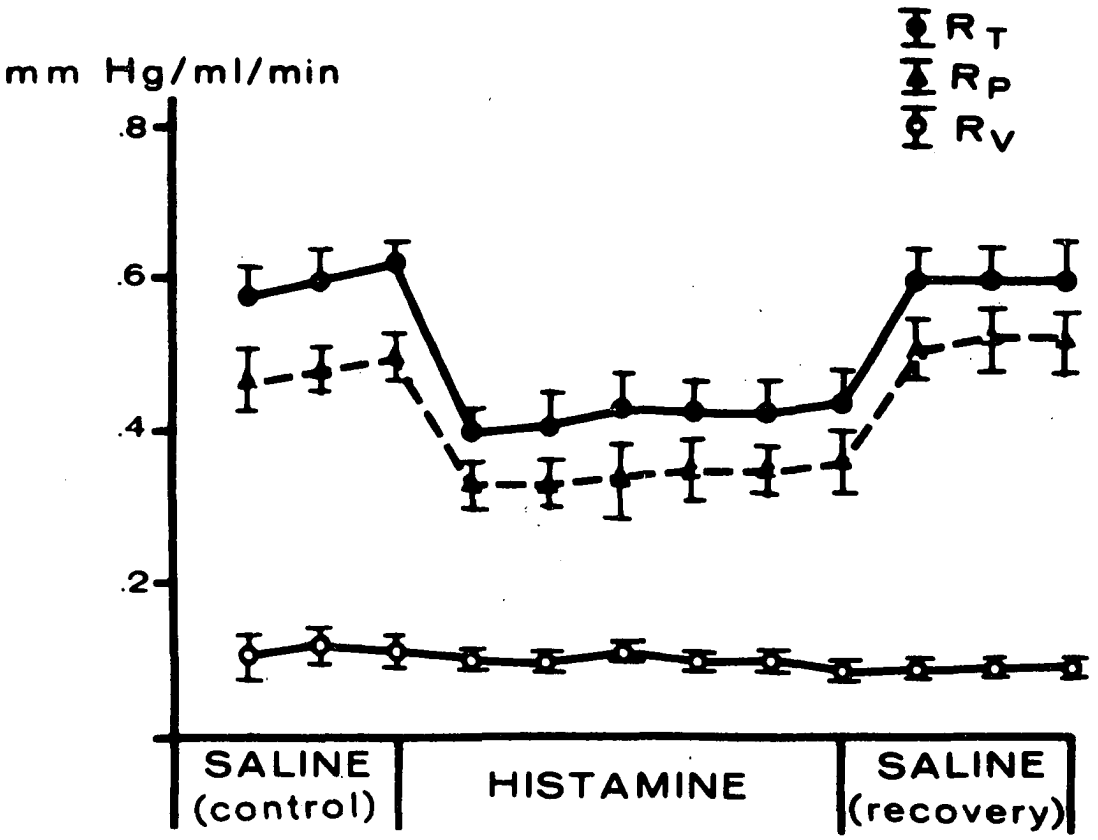

Figure 10. Total renal vascular resistance (R_t), prevenous resistance (R_p) and venous resistance (R_v) before, during and following renal arterial infusion of histamine. Calculated from observations at five min intervals. Means and standard errors for five animals shown.

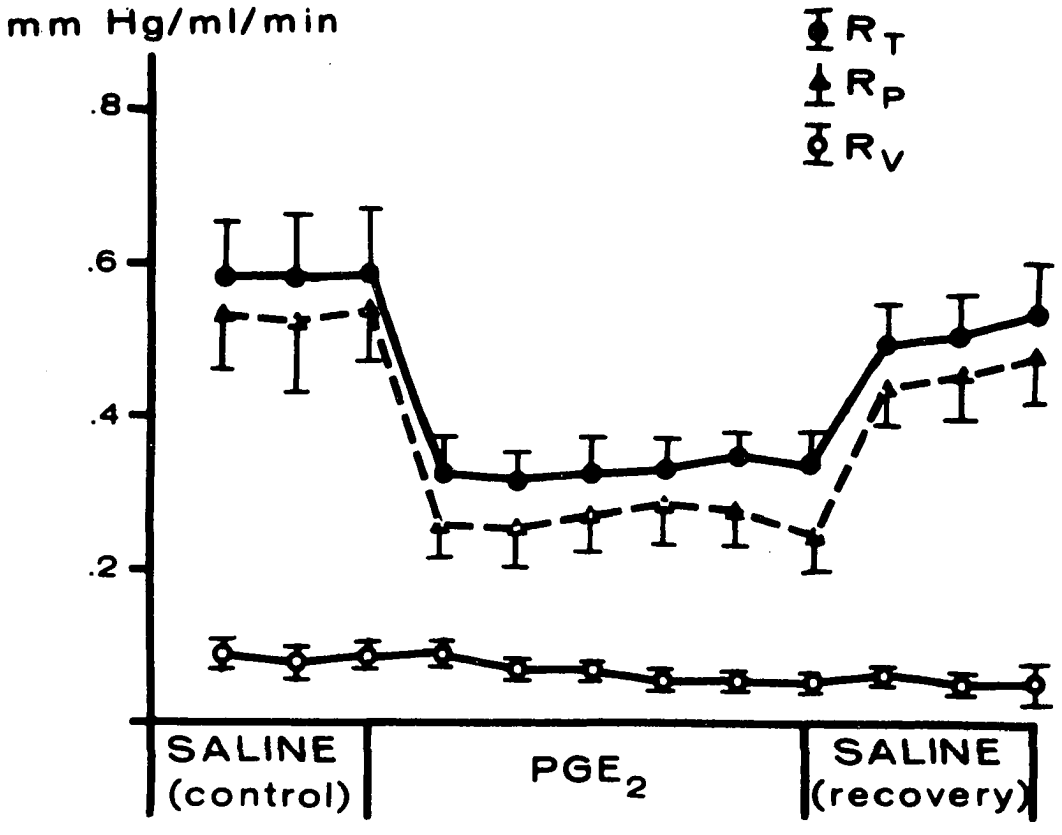

Figure 11. Total renal vascular resistance (R_t), prevenous resistance (R_p) and venous resistance (R_v) before, during and following renal arterial infusion of PGE_2. Calculated from observations at five min intervals. Means and standard errors shown for five animals (R_t) and four animals (R_p, R_v) shown.

of 19 mm Hg, as shown in Figure 7. PGE_2 also increased SCP significantly from an average 18 mm Hg to a mean 25 mm Hg during the experimental period (Figure 9). There is no significant difference in SCP changes between the two groups.

Renal Lymph

Hilar Lymph Flow

Figure 12 illustrates a significantly increased hilar lymph flow with histamine infusion. An average control flow of 25 µl/min increased to a mean 37 µl/min during the infusion period. PGE_2 also changed hilar lymph flow significantly from a mean control value of 37 µl/min to an average experimental value of 49 µl/min (Figure 13). There was no statistical difference between either control or experimental lymph flow values between the two groups. Flow returned to control values immediately upon termination of the drug infusions.

Capsular Lymph Pressure

Capsular lymph pressure (L_cP) increased significantly from 6 mm Hg to an average 10 mm Hg during histamine infusion (Figure 7). PGE_2 (Figure 9) significantly changed L_cP from an average 6 mm Hg to a mean experimental value of 11 mm Hg. Again, there was no significant difference in L_cP elevations between the histamine and PGE_2 groups.

Hilar Lymph Protein

Hilar lymph to arterial plasma ratios for protein (L_H/P) averaged 0.30 during the histamine (Figure 12) experiments and 0.33 during the PGE_2 (Figure 13) experiments. L/P ratios for total protein did not change with either drug, indicating that lymph protein concentration did

not change. Additionally, there was no difference in L/P ratios between the two experimental groups.

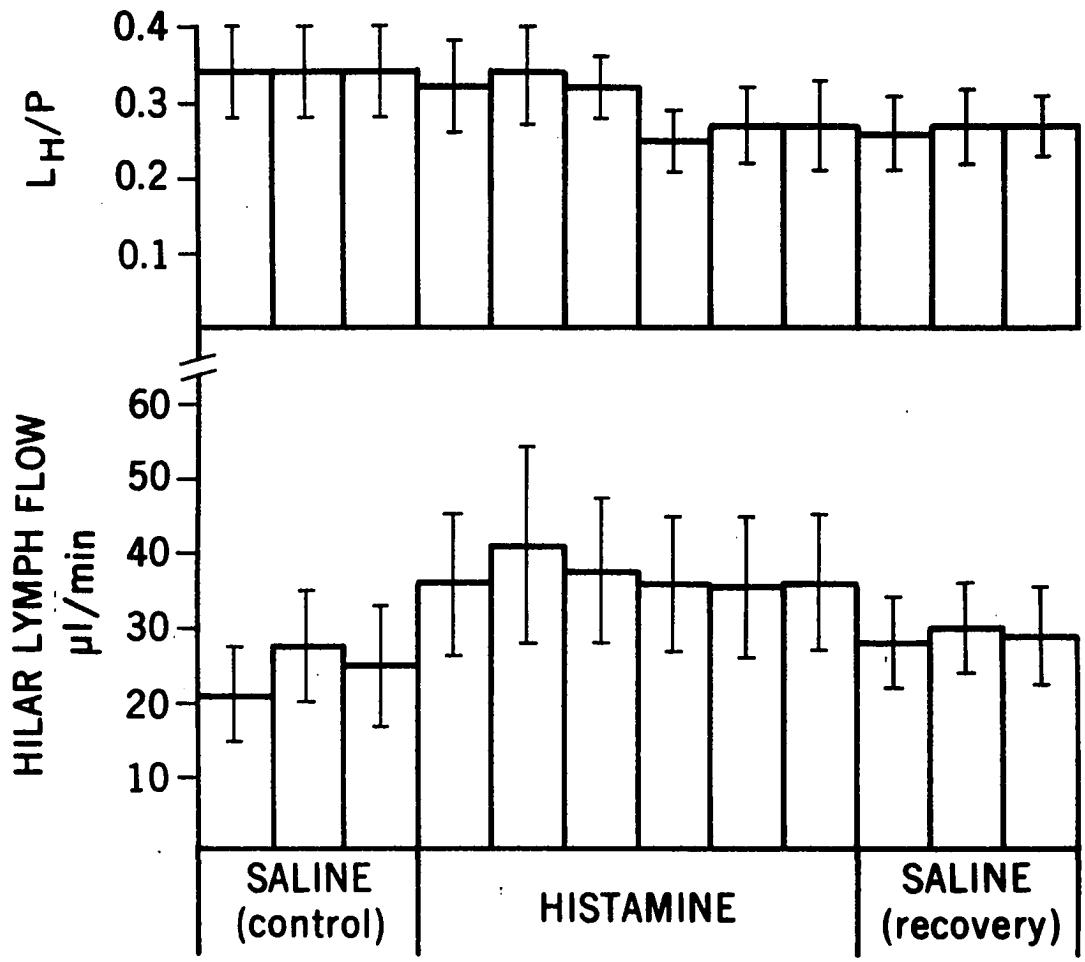

Figure 12. Hilar lymph to arterial plasma (L_H/P) ratios for total protein concentration are shown at the top and hilar lymph flow in ul/min at the bottom before, during and after renal arterial infusion of histamine. Each observation made at five min intervals. Means and standard errors for five animals shown in each graph.

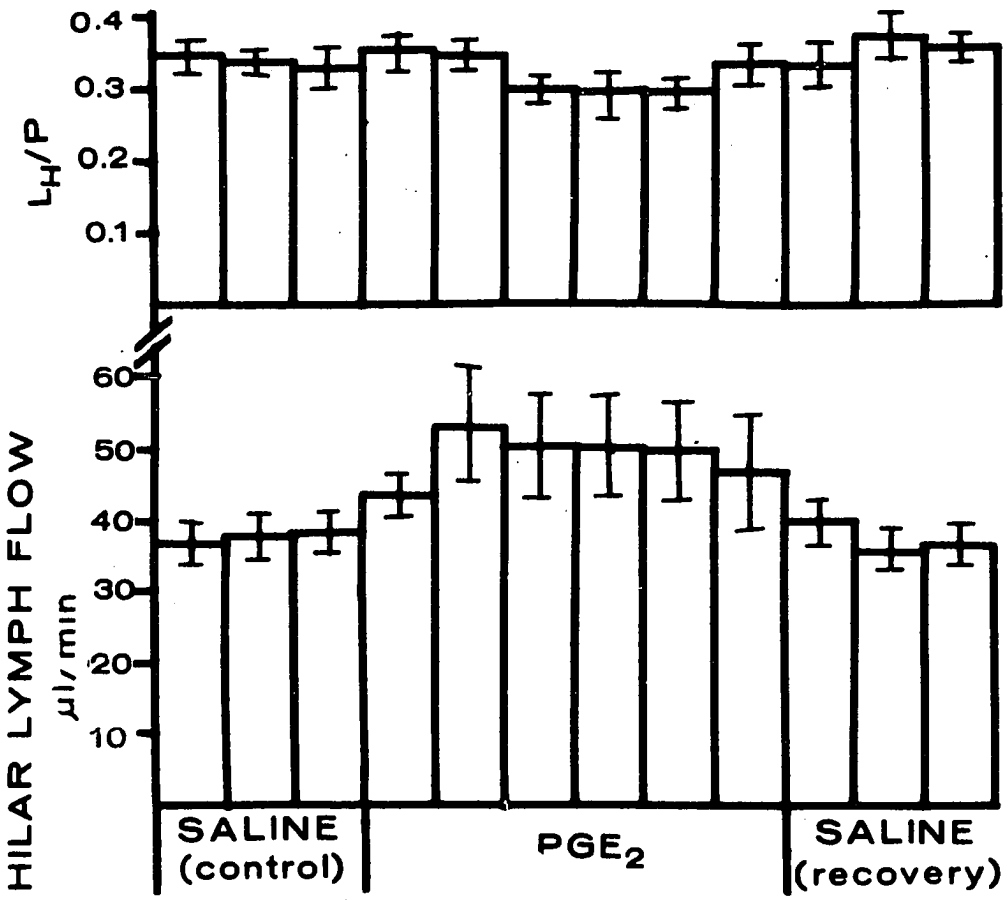

Figure 13. Hilar lymph to arterial plasma (L_H/P) ratios for total protein concentration are shown at the top and hilar lymph flow in ul/min at the bottom before, during and after renal arterial infusion of PGE_2. Each observation made at five min intervals. Means and standard errors for five animals (L_H/P) and six animals (lymph flow) shown.

CHAPTER IV

DISCUSSION

This research was designed to study the effects of two naturally occurring vasoactive agents, histamine and PGE_2, on renal fluid dynamics, renal lymph flow and protein composition. Both agents have been implicated in local control of blood flow. The most recent theories suggest that prostaglandin is intimately related to renal autoregulation, natriuresis and the pathogenesis of hypertension. Histamine may or may not have a physiological role in blood flow regulation but has been used extensively as a tool in cardiovascular pharmacology and physiology. Additionally, histamine has classically been reported to alter microvascular permeability to protein in such pathological states as anaphylaxis, allergy, injury and shock. In the course of these earlier investigations, histamine has been suggested as a renal vasoconstrictor and anti-diuretic principle. There is no information available on the effects of close renal arterial infusion of histamine, eliminating systemic effects and giving some specificity to the resulting action. Likewise, the effects of prostaglandin on the factors which may control lymph production in the kidney have not been studied.

The in situ canine kidney preparation of Bell (13) was adapted to the study of the above problems by developing methods by which microsamples (20 µl) of renal lymph and arterial plasma could be collected

and added to the reagent with minimal delay in order to show changes within a 5 minute period and to prevent evaporation of the small samples. These data were analyzed along with changes in renal blood flow, urine flow and urine concentration, renal tissue pressure, lymph pressure and renal venous pressures.

Histamine

Renal Fluid Dynamics

The earliest investigations on the physiological effects of histamine were studied by subcutaneous, intravenous and intra-arterial administration, however, the I.V. route was primarily used. Dale and Laidlaw (57) concluded that the general vasodilation caused by histamine (Histamine Shock) is not shared by the kidney vasculature, which constricts. Urine flow decreased following the direction of the blood pressure.

Figure 1 illustrates the increase in RFB when histamine (3 μg/kg/min) was infused directly into the renal artery. Blood flow increased significantly and remained elevated until the cessation of the infusion, at which time the values returned to control. Mean arterial pressure did not significantly change (Figure 6). Urine flow, however, did increase significantly but returned to control values after the histamine infusion. Urine osmolality decreased during the infusion but then gradually returned to control during the recovery period (Figure 4). These results are at variance with previously reported data.

Morimoto (94), in 1928, injected low (5×10^{-5} - 5×10^{-9} mg) and high (1 mg) doses of histamine into the renal arteries of dogs and cats. The preparation he used required the ligation of the abdominal

aorta below the renal artery and drug infusion by means of a t-tube in the renal artery or via the contralateral renal artery. Blood from the renal vein was by-passed to the jugular and could be measured directly by a drop counter.

He found strong constriction of the renal vasculature regardless of dose. Mean blood pressures averaged 70-100 mm Hg, probably resulting in reflex sympathetic activity which constricted the renal vessels. The low systemic pressure was probably from the trauma of the extensive surgery required for this preparation as well as the direct effects of histamine.

Subcutaneous histamine given patients by Bjering (95) resulted in decreased systolic and diastolic pressure, urea and creatinine clearances. Reubi and Futcher (96) administered subcutaneous histamine to normotensive and hypertensive patients and found a decreased renal plasma flow in both groups.

Blackmore, et al (97) administered intravenous histamine to unanesthetized dogs and measured GFR, RBF and filtration fraction. He administered a constant I.V. infusion of 2.5 μg/kg body wt/min for 2 hrs. GFR remained constant but increased above normal following cessation of the histamine infusion. RBF significantly increased and remained elevated for one hour following the histamine infusion while filtration fraction decreased only during the infusion. Mean arterial pressure, recorded directly from the femoral artery, decreased from an average value of 115 mm Hg to about 80 mm Hg. Urine flow decreased considerably as a result of histamine infusion.

Dale and Laidlaw (57) and Morimoto (94) reported decreased RBF and urine flow. The high histamine dose level, the impurity of the early

drug and possibly surgical trauma no doubt result in increased reflex sympathoadrenal discharge with concomitant renal vasoconstriction. In hypotension and shock the renal vasculature constricts via renal sympathetic nerves and circulating epinephrine (207). Blackmore, et al (97) concluded that the decreased urine flow in their conscious preparation is due to an antidiuretic property of histamine. They reported a 30-35 percent blood flow increase, comparable to our findings, but a 30-40 percent decrease in urine flow. Further, they showed a 30 percent decrease in arterial pressure which must certainly evoke reflex sympathetic renal vascular constriction with a decreased RBF. Circulating histamine would, at the dose level used, continue to dilate the renal vascular bed. This decrease in resistance and increased blood flow should result in increased urine flow in these moderately hydrated dogs (15 ml/kg body wt). Later studies by Blackmore and Cherry (208) on normal dogs and dogs with surgically induced diabetes insipidus demonstrated that the antidiuretic response was related to release of ADH. Dogs with experimental diabetes insipidus did not respond to I.V. histamine infusion (2.5 μg/kg/min) with antidiuresis. It is well known that ADH blood levels are elevated by hemorrhage or pain and excitement (209) so it is not unlikely that the hypotension reported by Blackmore, et al (97) released ADH by reduced left atrial distention and carotid-aortic baroreceptor stimulation in addition to the above mentioned mechanisms. The action of high titers of ADH on the distal nephron could explain the phenomenon of decreased urine flow with increased RBF in Blackmore's preparation. There is no other evidence in the literature for antidiuretic effect of histamine other than indirectly from autonomic reflex mechanisms.

The technique used in our studies of infusion directly into the renal artery allowed adjustment of the drug dosage to a point where no significant systemic effects occurred. The renal vascular response to histamine infusion, in this preparation, was increased renal blood flow and urine flow with a significantly decreased total renal resistance and prevenous resistance. Renal venous resistance did not significantly change (Figure 10). The change in renal vascular resistance resulting from the renal arterial infusion of 3 μg/kg/min of histamine was entirely prevenous. Figure 10 illustrates the significant drop in R_t and R_p while R_v does not change, in contrast to similar studies on the dog's leg (83 - 85, 210). Haddy (83) reported that 2-14 μg/min histamine IA in the dog foreleg increased small venous pressure but did not change tissue (needle) pressure. Above 14 μg/min IA, edema became apparent whenever SVP exceeded 26 mm Hg. SVP did not rise, however, when blood flow was held constant and at low rates of histamine infusion (5 μg/min). Higher rates of infusion (30 μg/min) caused SVP to rise in the constant flow preparation. He suggested that low concentrations of histamine raise small vein pressure by arteriolar dilation (increased blood flow against unchanged venous outflow resistance) while large doses constrict the venous side by direct action on venous smooth muscle and indirect effects of sympathetic reflex stimulation elicited by a drop in blood pressure.

In contrast, Diana, et al (84) and Diana and Kaiser (85) infused low (1-5 μg/kg/min) and high (20-60 μg/mg/min) doses of histamine IA into the isolated dog hindlimb and found both arteriolar and venous dilation, regardless of dose. The entire vascular bed of the hindlimb was dilated by histamine. In a constant pressure preparation flow increased and in a constant flow preparation the arterial pressure fell, indicating an

increase in resistance vessel caliber. Histamine produced venodilation regardless of dose while postcapillary resistance was measureably reduced.

In the *in situ* dog kidney SVP significantly increased by an average 27 percent, from 25 mm Hg to 32 mm Hg. Mean RVP and R_v did not change during the course of the experiments in contrast to a decreased venous pressure and R_v in the isolated dog hindlimb (85). Only R_t and R_p decreased in our preparation with increased RBF acting against an unchanged venous resistance. Hence, SVP abruptly increased following closely the direction of the RBF (Figure 7). Haddy (83) found that at low doses histamine increased SVP by acting directly on the venous smooth muscle in an opposite manner to the arteriole. In our preparation there was no measureable effect on the renal venous bed at 3 µg/kg/min histamine other than the increased SVP described above.

RBF increased in this preparation due to decreased R_p. This increased RBF met an unchanged venous resistance and resulted in an increased SVP but an unchanged RVP and R_v. The unchanging R_v suggests an absence of direct or indirect effects of histamine on the venous smooth muscle and on the connective tissue stenoses at the arcuate-interlobar venous junctions, as demonstrated by Koester, *et al* (33). This might be a homeostatic mechanism, unique to the kidney, to allow full functional distention and maintenance of the high intrarenal pressure, possibly of importance for efficient filtration and reabsorption.

This high renal "tissue pressure" was estimated by subcapsular pressure (SCP), or, that pressure exerted by the renal parenchyma on a miniature, wafer-type transducer introduced between the kidney parenchyma and the relatively inelastic capsule. Hebert and Arbus (211)

reported that increases in deep renal venous pressure were accompanied by equivalent increases in SCP, except during saline loading. Additionally, Bell, et al reported that SVP (IRVP) is intimately related to renal tissue pressure and renal lymph pressure. When the renal artery was occluded for a short time TP, IRVP and L_cP decreased. Upon RBF reinstatement IRVP returned to control first, followed by TP and L_cP. The secondary return of TP and L_cP indicated that these factors are related to the filling of the interstitium depleted of fluid by the arterial occulsion and the return of IRVP. Changes in IRVP were reflected directly as changes in TP and L_cP (14). Hinshaw had earlier (45) found an intimate relationship between tissue pressure (needle pressure) and IRVP (SVP) and suggested that tissue pressure was a prime determinant of renal autoregulation.

In the dog leg, low rates of histamine infusion do not increase limb weight (84, 85) or only transiently increase limb weight which then decreases below control (210). These kinds of data suggest that, in the dog's leg, the entire vascular resistance is lowered so that the transmural pressure is decreased with subsequent net movement of interstitial fluid into the vascular space. The kidney, however, responded to histamine infusion with an elevated SCP because of vasodilation and increased vascular and interstitial filling. The SCP did not return to control until the termination of the histamine infusion but did decrease slightly (not significantly) as the increased initial load of urine and lymph left the kidney. Transmural pressure in the kidney vasculature must increase rather than decrease, as in the dog leg, since SCP, L_cP and lymph flow all become elevated. Again, the increased RBF acting against an unchanged venous outflow resistance resulted in elevated

vascular transmural pressure and subsequent filtration into the interstitium, reflected in SCP, L_cP and lymph flow.

Renal Lymph

Classically, histamine is a substance which increases the rate of transvascular fluid movement from plasma to tissue, regardless of route of administration (89, 212). Haynes (213) injected histamine (26 - 93 µg/kg/min) intravenously and intra-arterially into the dog leg and found an increased subcutaneous lymph flow with increased lymph protein concentration. Edery and Lewis (214) injected 1 mg histamine into the dog femoral artery which increased hindlimb lymph flow 3 to 6.3 times resting flow. They did not measure lymph protein concentration after histamine but concluded that the increase in lymph flow after injury was due to endogenous histamine release. Sturmer found later (215) that infusion of histamine into the dog femoral artery did not increase hindlimb lymph flow at a dosage rate of 1 µg/kg/min.

In 1970 Lewis and Winsey (216) infused histamine into the cat hindlimb at 3 µg/min. They reported a 190 percent blood flow increase with a 165 percent increase in hindlimb lymph flow. Lymph protein concentration increased 53 percent. The authors concluded that histamine was quite potent in elevating lymph flow but that blood flow increases are not enough to elevate lymph flow since prostaglandin, a potent vasodilator, did not increase lymph flow very much. Haddy, et al (217), in 1972, found that histamine infused IA (3 - 40 µg/min) in the dog forelimb increased femoral lymph protein concentration as well as increasing flow as Haynes (213) had found earlier. In general, histamine increased lymph flow and lymph protein concentration in the dog or cat

leg except at very low doses.

In our experiments (Figure 12) renal hilar lymph flow significantly increased by 50 percent when histamine was infused IA. Immediately upon cessation of the histamine infusion lymph flow returned toward control. Hilar lymph to arterial plasma ratios for protein did not change, however, at any time during the experiment. Capsular lymph pressure (L_cP) increased about 75 percent reflecting the increased lymph production and flow in addition to the increased renal tissue pressure (SCP).

No significant change in lymph protein concentrations (relative to arterial plasma) suggested that histamine, in the dosage used, did not alter membrane characteristics so as to allow the movement of large protein molecules from the plasma to the interstitium to be removed by the lymphatics. The striking effects of histamine on the venular endothelium of the rat cremaster (90) and other organs (92) could not be demonstrated in the kidney (93). Schwartz and Cotran suggest that since the kidney has few mast cells the renal vessels may not respond to histamine (93). The brain and testis also have few mast cells and do not respond to the vascular-labeling type of experiment with histamine (92). While the venules of the rat kidney did not, apparently, respond to histamine the dog renal arteriolar bed did. The above results and discussion demonstrate that IA histamine lowers renal vascular resistance with secondary increases in lymph and urine flow.

Diana, et al (84) suggested that high dosage rates of histamine (20 - 60 μg/kg hindlimb/min) alter membrane permeability as an explanation for histamine edema. Since the entire vascular bed of the hindleg is dilated, elevated capillary or venular pressure could not be the cause

of increased filtration and edema. If the concept that increased vascular permeability results in an increased transfer of protein molecules (albumin) from the plasma to the extravascular space is acceptable (218), then the intra-arterial infusion of large doses of histamine into the kidney does not alter vascular membrane permeability, as reflected in lymph to arterial plasma ratios for protein.

The question remaining, then, is that if the increased SCP, L_cP and lymph flow did not represent increased vascular permeability, what did they mean?

Sabin (219) defined a lymphatic as a "modified vein" and presented convincing evidence that lymphatics are embryologically derived from veins. This is of interest since Bell, et al (12) feel that changes in lymph formation with increased RVP (11) and the fact that alterations in renal perfusion pressure have no effect on lymph production (47) point to the renal venous system as the primary source of renal lymph production. Thus, there may be a functional relationship between renal lymph production and the renal venous system still requiring clarification.

Histamine, then, did not alter vascular permeability to protein in the kidney, as reflected by lymph protein concentration, as it did in the periphery. The arteriolar bed of the kidney was dilated while no direct effect on venous resistance was seen although small vein pressure increases. The increased blood flow resulted in a greater urine production by possibly washing out medullary osmolar gradients.

Prostaglandin E_2

Renal Fluid Dynamics

Renal arterial infusion of 0.4 μg/kg/min of PGE_2 increased RBF by an average 75 percent above control (Figure 2). Higher infusion rates resulted in undesirable systemic depressor responses. Single injections of 0.56 - 3.2 μg/kg PGE_2 (220) and I.V. infusion of 0.25 - 4.0 μg/kg (221) decreased systemic pressure with increased heart rate, probably a reflex reaction (166). PGE_2 was equally active to PGE_1 (220). I.V. administration of PGE_1 (1 μg/kg) lowered systemic pressure by dilating the splanchnic bed while renal and femoral blood flows were unchanged (222). The mechanism by which prostaglandin dilates arterioles is unknown but is both postsynaptic and myogenic (223).

Very low dose levels of PGE_1 or PGE_2 (0.01 μg - 5.0 μg) (187 - 189, 224) greatly increased RBF. Cortical blood flow increased while outer medullary flow fell which also occured with ethacrynic acid, furosemide and acetycholine (225). Since filtration fraction decreased with increased renal plasma flow (GFR did not change) (187, 188), the prostaglandins probably preferentially act on the efferent arteriole.

In our preparation urine flow increased almost four times (Figure 5) while urine osmolality decreased by over half. The urine flow change was reversed when the infusion was terminated but osmolality gradually returned to control levels. These findings are in agreement with Shimazu, et al (189) who reported that urine flow tripled at a PGE_1 infusion rate of 0.2 μg/kg/min in addition to decreased urine osmolality. Further, Lee reported that renal IA infusion did not result in increased urine flow from the contralateral kidney (224).

PGE (and PGA) compounds rank among the most potent natriuretic factors (222). Johnston, et al (187) and Vander (189) reported greatly increased sodium and potassium excretion with PGE_1 or PGE_2 IA infusion while GFR remained stable. Osmolar clearance and free water clearance increased also, suggesting PG inhibition of sodium reabsorption and anti-diuretic hormone-like action. PGE_1 diminished the permeability response of toad bladder (226) and isolated rabbit collecting tubule (227) to vasopressin. The mechanism by which prostaglandins inhibit tubular sodium reabsorption is not understood, but it is known that physical changes in the postglomerular circulation lead to natriuresis and diuresis (228 - 230). Such a change may be increased peritubular capillary blood flow and pressure resulting from a sodium load or various vasodilating drugs. Since PGE and PGA preferentially dilate the efferent arteriole, this may serve to increase peritubular capillary blood flow and inhibit sodium reabsorption.

Our results on RBF, urine flow and urine osmolality are in complete agreement with other investigators using the same type of preparation (187 - 189, 224). This kind of consistent result among many laboratories has led to at least three theories of the physiological role of prostaglandin, with regard to the kidney.

Lee (222) suggested that the renomedullary prostaglandins are the natriuretic hormone. The evidence in support of this hypotheses is that prostaglandins are normally found in the renal medulla and that these compounds mimic the effects of saline loading on the kidney. These effects have been discussed above. The two other theories are that prostaglandin is an antihypertensive hormone (222) and that these compounds act as an intrarenal regulator of renal autoregulation (200).

Prostaglandin must be shown to be an intrarenal controller of blood flow to prove the above hypotheses.

Intra-arterial PGE_2 infusion resulted in both increased SVP and SCP (Figure 9) which returned to control upon cessation of the infusion. These parameters and segmental renal resistances have not previously been studied under the influence of PGE_2 in the *in situ* canine kidney.

Daugherty (231) reported that, in the dog forelimb, IA PGE_1 decreased total vascular resistance and increased blood flow. Muscle and skin venous resistance also fell over the dosage range used (0.5 - 10 µg/min). This is in contrast to our findings in the kidney where only R_t and R_p fell significantly (Figure 11). Daugherty's dose levels of PGE_1 were similar to those of ours for PGE_2.

Greenberg and Sparks (232) studied the vascular response to IA PGE_1 and PGA_1 (0.01 - 10 µg/min) in the dog forelimb. Vascular resistance decreased with both prostaglandins while capacitance, as reflected in limb weight, increased. "Capacity" increased in both natural and constant flow preparations. Additionally, isolated femoral venous smooth muscle relaxed in the presence of PGE_1 and PGA_1. The authors concluded that prostaglandin was unlike other vasodilating drugs in that it had this "capacitance-increasing" effect.

In our *in situ* kidney preparation, R_v (Figure 11) and RVP (Figure 8) did not change with PGE_2 infusion. SVP (Figure 9) increased, however, reflecting the increased RBF acting against the unchanged resistance of the renal venous stenoses described previously. There is no evidence for any direct action of PGE_2 on the renal veins but the high SVP may have obscured and R_v changes. Additionally, the very high levels of prostaglandin dehydrogenase and reductase in the cortex may have deactivated

most of the PGE_2 before it reached the renal veins.

Both Daugherty (231) and Greenberg and Sparks (232) could find no evidence that capillary filtration increased with prostaglandin infusion when measured by changes in leg weight. Comparable doses in the in situ kidney resulted in a significant increase in SCP (Figure 9). This increase in the turgor of the renal tissue was the result of many factors, including increased vascular and tubular filling. Increased plasma filtration occured also and is reflected in increased lymph flow and pressure as well as increased SCP.

Thus, PGE_2 increased RBF by lowering arteriolar resistance. Urine flow is increased due to increased blood flow and poorly understood factors inhibiting the tubular reabsorption of sodium, while the osmolar concentration of the urine decreases. The increased RBF acts against an unaltered venous resistance reflected as elevated SVP. It has been shown (14) that elevations of SVP will be followed by equivalent increases in SCP and L_cP. The kidney was then unlike the dog's leg (231, 232) in that PGE_2 increased filtration of fluid from the renal vasculature. This was evidenced by changes in the production of renal lymph.

Renal Lymph

Our data confirm the results of Fujimoto and Lockett (185) with respect to lymph flow. Renal hilar lymph flow did increase by an average 31 percent while L_cP increased by 114 percent. Pressure changes were greater than flow changes probably because the hilar lymphatics are an anastomotic network through which increased flow and pressure might equalize somewhat. The capsular lymphatic, however, does not anastomose in that manner, so that the increased L_cP, secondary to increased lymph

production was presented to the pressure catheter. In addition, the small diameter tubing used to measure the lymph flow introduced a high resistance into the flow path.

Hilar lymph to plasma ratios (L_H/P) for total protein were unchanged under the influence of PGE_2. Weiner and Kaley (233) and Willoughby (234) found increases in vascular permeability with PGE_1 in rat skin and rat cremaster muscle. PGE_1 was even more effective than histamine or serotonin at equal dosage rates. Further, cat hindlimb lymph did not increase in protein concentration when IA PGE_1 was infused 1 - 20 μg/min (216).

As with histamine, PGE_2 did not, under the conditions of these experiments, alter vascular properties such that large molecules left the plasma and entered the lymph (via the interstitium) in a manner different than during control periods. While lymph flow and pressure often dramatically increased, the composition with respect to protein concentration remained the same. The kidney and the leg reacted in the same manner to E prostaglandins in contrast to histamine where leg lymph flow rate and protein concentration were not independent of each other.

Lymph flow was found to not change, however, in the cat hindlimb during IA infusion of 1 - 20 μg/min PGE_1 by Lewis and Winsey in 1970 (216). Blood flow did increase however, perhaps adding weight to the findings of Daugherty (231) and Greenberg and Sparks (232) that the dog leg vasculature was entirely dilated without any evidence for increased weight (edema formation). Additionally, increased venous pressure along increased cat hindlimb lymph flow.

Renal lymph flow in cats was studied by Fujimoto and Lockett (185). These workers found that norepinephrine, angiotensin II and PGE_1

increased renal lymph flow when infused intra-aortically. Renal lymph was collected from the cisterna chyli rather than directly from renal lymphatic trunks which the authors mentioned seeing. Mesenteric inputs were ligated, supposedly leaving only input from renal lymphatics. PGE_1 (0.13 - 0.19 μg/kg/min) and the other vasoactive agents were infused into the aortic arch. Urine flow and urine sodium excretion rose but filtration fraction was unchanged, in contrast to the findings of Johnston, et al (187) and Vander (188) who found an increased filtration fraction in dogs.

Histamine and PGE_2 Compared

On the basis of dosage rates of histamine and PGE_2 which did not, on the average, alter mean arterial pressure more than about ± 5 mm Hg it was concluded that the two agents were similar with respect to the parameters studied. Of course, on the basis of weight, PGE_2 was 7.5 times more potent in eliciting the same fluid dynamic changes.

RBF was found to be higher with PGE_2 than histamine while average control flows were not significantly different (Figure 3). Interestingly, renal vascular resistances were not significantly different between the histamine and PGE_2 experimental groups. While mean arterial pressures between the two groups were not significantly different, two animals of the PGE_2 group averaged control mean arterial pressures of 160-165 mm Hg which changed several hemodynamic factors. RBF will be higher at a given resistance because of the higher driving force, or perfusion pressure. For this reason it was concluded that the PGE_2 RBF was higher than histamine RBF because with resistance constant, RBF was proportional to perfusion pressure. Certainly, with renal autoregulation, resistance

is changing, making RBF relatively independent of perfusion pressure, in the autoregulatory range. In the case of the IA infusion of histamine or PGE_2, the resistance vessels were overcome by the drug and dilate to possibly a maximal degree.

With both drugs the increased RBF acted against the unchanging resistance of the renal venous stenoses described by Koester, et al (33). On the average, it seems as if this anatomical restriction to outflow is a consistent resistance. While RBF between the two groups was different, SVP, SCP and L_cP were not. SCP and L_cP have been shown to increase linearly with SVP elevations (14).

RBF could not be increased further with PGE_2 or histamine without general sympathoadrenal discharge. Not only would sympathetic stimulation antagonize the action of the vasodilating agents used, but the sympathomimetic agent norepinephrine has been reported to increase renal lymph flow itself (185).

Freie and Bell (51) found that the renal IA infusion of 0.01 mg/kg/min acetylcholine dilated the prevenous segment of the renal vasculature with no change in venous outflow resistance. As with histamine and PGE_2, acetylcholine infusion increased SVP, followed by increased SCP and L_cP in a directly related manner. Neither capsular nor hilar lymph to arterial plasma ratios for protein changed significantly with acetylcholine infusion (13).

The results of these experiments indicate that the prime determinant of renal lymph flow changes was changing RBF acting against an unaltered venous outflow resistance. Intra-arterial infusion of histamine, PGE_2 or acetylcholine (51) resulted in a prevenous resistance drop and increased RBF. SVP increased while the arterial pressure transmitted

through the decreased prevenous resistance increased the vascular transmural pressure and subsequent filtration into the extravascular space.

Histamine may not play a physiological role (63). It is, however, a useful tool in altering renal hemodynamics but is no better than acetylcholine and probably more toxic. Prostaglandin, on the other hand, is strongly implicated in the kidney as the agent responsible in controlling intrarenal hemodynamics, sodium excretion and blood pressure. Lee (222) has postulated that the mechanism whereby prostaglandin travels to the renal cortex from its points of biosynthesis in the medulla is via vasa rectae or lymphatics. The mechanism of natriuresis and diuresis would be increased peritubular capillary pressure inhibiting the tubular reabsorption of sodium. The results of the research reported here are consistent with this mechanism since SVP dramatically increases and is probably transmitted upstream to the capillaries.

Histamine and acetylcholine (51), however, acted in the same manner by increasing SVP with subsequent diuresis and probably natriuresis. PGE_2, in these experiments, exerted no unique action on the renal vasculature not done by histamine or acetylcholine. The mechanism by which prostaglandin is transported to the cortex is questionable, also. There is very little evidence (7) for medullary lymphatics communicating with the cortex or even existing at all. Transport via the vasa rectae is quite possible but required elucidation. Tubular transport of prostaglandins is questionable because high concentrations of prostaglandin degrading enzymes are found in the ascending thick limb of the loop of Henle (132).

Certain other aspects of the relation of renal fluid dynamics and lymph flow to vasoactive agents should be investigated next.

First, Fujimoto and Lockett reported that norepinephrine increased renal lymph flow in their preparation (185). Would I.A. or I.V. norepinephrine increase renal lymph flow in the in situ dog kidney? What might be the mechanism in view of the fact that vasodilating agents dilate the prevenous segment while norepinephrine must constrict this same bed?

Second, histamine and PGE_2 have been reported to alter vascular permeability but did not do this in the kidney. The ideal preparation would be an isolated perfused kidney in which much larger doses of the agents could be given without sympathetic reflexes complicating the hemodynamic response. Would lymph protein concentration then be altered?

Third, PGE_2 is known to shift renal blood flow from the outer medulla to the cortex. Could PGE_2 and other vasoactive agents shift RBF in such a way that lymph composition could reflect the physiology of the different cortical areas?

CHAPTER V

SUMMARY AND CONCLUSIONS

Canine renal lymph was collected from hilar lymph vessels before, during and after the renal arterial infusion of systemically sub-hypotensive doses of either histamine diphosphate or prostaglandin E_2. Lymph flow rate and capsular lymph pressure were measured while hilar lymph and arterial plasma were analyzed for total protein concentrations.

Renal blood flow was measured by an electromagnetic flowmeter in addition to the recording of renal venous pressure (RVP), renal small vein pressure (SVP), sub-capsular pressure (SCP) and mean arterial pressure (AP). Urine flow rate and osmolality were also determined.

Both histamine (3 μg/kg/min) and PGE_2 (0.4 μg/kg/min) decreased R_t and R_p with no effect on R_v. RBF increased, transmitting greater hydrostatic pressure against an unaltered venous outflow resistance which caused SVP to rise. SCP and L_cP increased secondary to SVP while lymph flow also increased. Lymph protein concentration, expressed as lymph to arterial plasma concentration ratios (L_H/P), remained stable and independent of lymph flow rate. Urine flow dramatically rose while osmolality decreased. All effects were reversed upon cessation of the drug infusion with a rapid return to control values.

This research allows the questions posed in Chapter I to be

answered. Namely:

1. Histamine does not alter vascular permeability to protein in the kidney. L_H/P ratios for protein were not significantly changed during the course of the experimental period. PGE_2 infusion does not change L_H/P protein ratios, either. It is concluded that lymph protein concentration is independent of lymph flow in the canine kidney under the influence of vasodilating drugs.

2. Renal intra-arterial infusion of histamine or PGE_2, in doses below those which bring about a generalized sympathoadrenal discharge followed by renal vasoconstriction, dilate the prevenous segment of the renal vascular bed. There is no measureable effect on renal venous resistance (R_v) or renal venous pressure (RVP). At these dosage levels PGE_2 administration elevates RBF and urine flow more than histamine, but this can be explained on the basis of higher perfusion pressure in some of the PGE_2 dogs. RBF acting against the unchanged venous outflow resistance increases SVP and, secondarily, SCP. This increased SVP transmitted upstream in addition to increased tubular and vascular filling results in elevated lymph production. The increased lymphatic outflow is reflected in increased L_cP. Urine flow greatly increases for the following reasons:

a. The osmolar gradient in the renal medulla could be "washed out" by increased vasa recta blood flow. The counter-current multiplier system would be ineffective.

b. The increased peritubular capillary pressure secondary to increased SVP inhibits the tubular reabsorption of sodium, followed by diuresis.

c. There could be a direct effect of the agents on the tubular cell to inhibit sodium and/or water reabsorption.

3. The physiological role of both histamine and prostaglandin is not clear. Histamine has been often implicated in many roles but may well be an evolutionary vestige (63). More recently, prostaglandin has been suggested as an intrarenal regulator of blood flow and sodium excretion as well as a systemic antihypertensive agent.

The present research has shown that, under the conditions of these experiments, both histamine and PGE_2 produce the same effects when infused intra-arterially. PGE_2 is about seven times more potent, by weight, but seems to exert no unique effects. Other vasodilating agents, such as acetylcholine (51) have been reported to have the same effect, qualitatively, in the kidney as PGE_2 or histamine. These agents, possibly by a common mechanism, dilate the arteriolar bed of the kidney. While PGE_2 appears to have the same effects as other agents in the kidney, it seems as if the remarkable potency of PGE_2 lends credence to the recent theories of its physiological role.

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