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VASOPRESSOR ACTIVITY IN RENAL VENOUS PLASMA AND RENAL HILAR LYMPH

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VASOPRESSOR ACTIVITY IN RENAL VENOUS PLASMA AND RENAL HILAR LYMPH

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CHAPTER I

INTRODUCTION

The carotid sinus has been shown to be the sensory element of a reflex arc that functions to regulate arterial pressure (35). The sinus is a pressostat. Sensing an increase or decrease in systemic blood pressure as an increase or decrease in wall tension, the carotid sinus initiates appropriate changes in sympathetic vasoconstrictor activity. Thus, the carotid sinus mechanism may be thought of as part of a central, neural mechanism for the regulation of arterial pressure. Recent investigations have suggested that perhaps a local baroreceptor also exists within the parenchyma of the kidney (41). While the carotid sinus promotes neural or neuro-endocrinological adjustments of the arterial blood pressure, the effect of stimulation of the proposed renal baroreceptor appears to elicit only an endocrine-like response.

The Renin-Angiotensin System

In the latter half of the 19th century Brown-Sequard began investigations of the physiological activity of extracts of various endocrine organs, notably the thyroid and testes. In an effort to extend Brown-Sequard's study of the "internal secretions" of the glands of the body, Tigerstedt and Bergman in 1898 extracted kidneys with saline (32).

Injection of these kidney extracts into rabbits resulted in a rise in the blood pressure of the animals. Since that time numerous studies have appeared that indicate that the kidney is the site of manufacture of vasoactive substances. The renin-angiotensin system is now well established as an enzyme system of renal origin which produces the most potent peripheral vasoconstrictor yet found (26, 39). Briefly, the system operates as follows: renin released from the kidney acts on an α-2 globulin of the blood plasma to produce the decapeptide, angiotensin I. Due to the action of a chloride activated enzyme in the plasma, the decapeptide is then cleaved to the vasoactive octapeptide angiotensin II. Once formed angiotensin II is susceptible to attack by angiotensinases in the plasma and tissue fluids. The angiotensinases hydrolyze the octapeptide and produce a mixture of non-pressor peptides (25, 30). The reactions involved in the formation and destruction of angiotensin II appear in Figure 1. Figure 1 also represents diagramatically the hypothetical routes of renin release into the renal venous blood and renal hilar lymph.

Since a pressor substance released by the kidney may be involved in the establishment of experimental or naturally occurring hypertension, it would be of value to understand the mechanism of the release of such a vasoactive substance (or substances) into the blood and lymph. At present the mechanism or mechanisms which control the release of renin from the kidney are not fully understood (1, 29, 41, 42, 47). Further, the question of whether the renal venous effluent or renal lymph serves as the principal route of renin release has been a subject of debate (17, 40). Indeed, until recently some investigators had been unable to

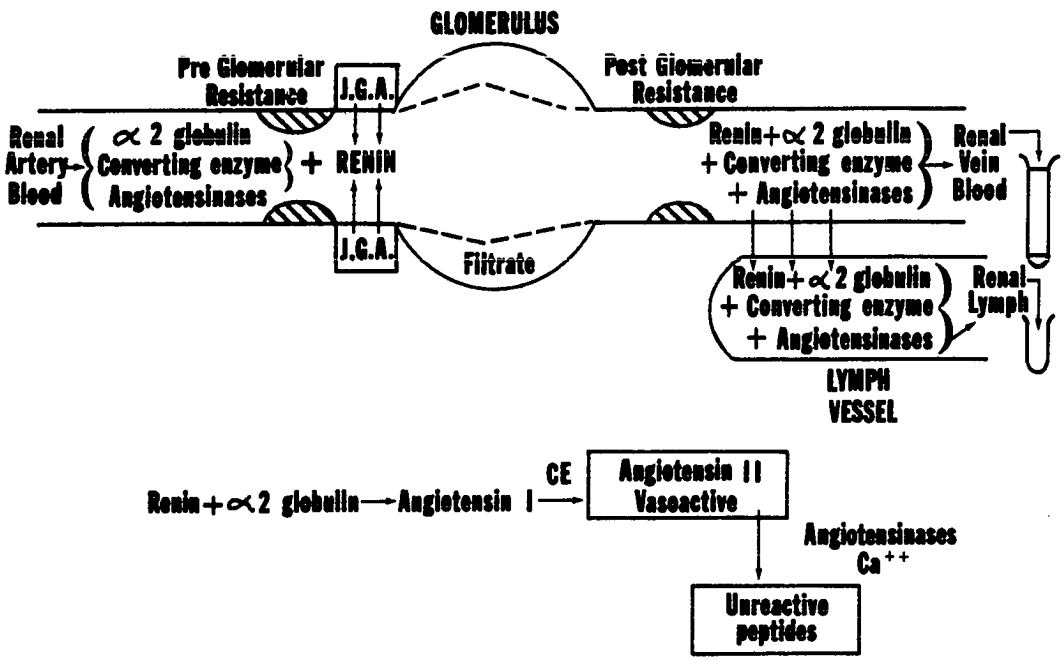

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Figure 1. Top. Hypothetical routes of renin release. J.G.A. = juxtaglomerular apparatus. Bottom. Formation of Angiotensin II. CE = converting enzyme.

demonstrate the presence of renin or angiotensin in the renal vein blood of either normal or hypertensive animals (18, 27). Failure to recognize that the renin-angiotensin system is, indeed, an enzyme system has been responsible for many of the contradictory findings present in the literature. To determine if renin is present in a certain body fluid, one must eventually perform a biological assay on the unknown sample. In the case of renin the unknown sample is most often injected intravenously into the anesthetized test animal, while the systemic blood pressure of the animal is continuously recorded. A rise in blood pressure of the test animal following the injection of a test sample is taken as a positive response for the presence of renin and/or angiotensin. It should be noted here that neither renin nor angiotensin I is considered vasoactive (10). Angiotensin II, the product of two enzymatic reactions within the system, is vasoactive and is the material which, if present in sufficient concentration, will produce an increase in blood pressure upon injection into an appropriate test animal.

In order to determine the presence of a biologically active substance in an unknown sample, one must have the unknown present in the sample in a concentration which will give the smallest acceptable response in the assay animal. The ability to detect an unknown substance in a biological assay, then, can be improved by either increasing the sensitivity of the test animal and/or by increasing the concentration of the unknown in the sample. If the substance of assay is the product of an enzyme reaction, the concentration of the active material in the test solution may be increased by producing one or more conditions which favor product formation. Such conditions would include incubation at

optimal temperature and pH, increasing substrate concentration, and increasing enzyme concentration (49). Further, if there are enzymes in the sample that attack the assayable product, inhibition of these would increase product concentration at the time of assay. Lastly, the concentration of the product can be increased by reducing the amount of solvent, i.e. concentrating the total sample.

The Vasoconstricting Activity of Renal Venous Blood

It will become apparent that much of the conflicting data presented by studies concerned with the renin content of renal vein blood is due to the different experimental approaches used in the studies. These different approaches affected either the sensitivity of the assay system or the concentration of angiotensin II in the assay samples.

In 1938 Fasciolo, Houssay and Taquini (5) grafted kidneys from dogs, made hypertensive by the method of Goldblatt, into the necks of normotensive animals. Five to ten minutes after completion of the graft the blood pressure of the normotensive recipient dogs rose 32-70 mm Hg. These authors concluded that the ischemic kidney produces a vasoconstrictor substance capable of producing sustained hypertension. In order to substantiate further this conclusion, renal venous plasma samples obtained from both normotensive and hypertensive animals were assayed for pressor activity using the Laewen-Trendelenburg toad preparation. At constant pressure, infusion of renal venous plasma obtained from hypertensive animals decreased the flow through the preparation 63% from the control value. In contrast, injection of renal venous plasma of normal dogs decreased flow only 2.8% from control values. These results supported the idea that the ischemic kidney secretes a vasoconstrictor

substance into the renal vein which is responsible for the development of the permanent hypertension seen in dogs with restricted renal blood flow.

Mason and Rozell (20) attempted to repeat the studies of Fasciolo, Houssay and Taquini. However, they were unable to demonstrate any difference in the vasoconstricting property of plasma taken from normotensive or hypertensive dogs. They inferred that the Laewen-Trendelenburg preparation was unsatisfactory for the assay of pressor substances in dog plasma and suggested that the varying stability and sensitivity of the assay bed was responsible for their inability to confirm the findings of Fasciolo, et al. In 1940 Kohlstaedt and Page (16) assayed samples of canine renal venous plasma for pressor activity. Samples were obtained before and after clamping the renal artery of an isolated pump-perfused kidney ventilated through an isolated lung. By appropriate adjustment of the pump output when clamping the artery, the pulse pressure of the isolated, perfused kidney could be decreased, while the mean perfusion pressure was maintained at control levels. Forty minutes after reducing the pulse pressure of the isolated kidney, a vasoconstrictor substance appeared in the renal venous effluent. Kohlstaedt and Page concluded that the reduction of the pulse pressure to the kidney stimulated renin release into the renal venous blood.

Two additional points should be mentioned in regard to the 1940 study of Kohlstaedt and Page; one relates directly to the experimental treatment of the kidney, while the other is concerned with the assay procedure. Throughout the experiment the isolated pump-perfused kidney was oxygenated via an isolated lung preparation ventilated with

95% O_2, 5% CO_2. However, no attempt was made to locally alter the oxygen tension of the kidney during the experiment. Further, the samples of renal venous plasma obtained from the experimental kidney were mixed with renin substrate just prior to assay in the rabbit ear. Increasing the substrate concentration should have increased the velocity of formation of angiotensin II in any given sample which may have increased the concentration of angiotensin II in the assayed samples.

In their study of the renin content of the renal venous plasma of dogs before and after constriction of the renal artery, Dell'Oro and Braun-Menendez (3), subjected the plasma to several procedures designed to increase the angiotensin content of the sample. These procedures included acidification, incubation at 37° for 2 hours, addition of bovine plasma and alcoholic extraction. Acidification would inhibit angiotensinase, while addition of bovine plasma should increase renin substrate. Incubation increases the velocity of the formation of angiotensin II and extraction would concentrate the assayable angiotensin in the sample. The extracts were assayed by intravenous injection into dogs and the pressor effect, if any, was monitored. These authors found no renin in the samples of renal venous plasma obtained prior to the application of the Goldblatt clamp. However, following partial constriction of the renal artery, renin appeared in samples of both renal venous and femoral venous blood. Further, extracts of renal venous plasma elicited a greater pressor response in the assay animal than did the corresponding extracts of femoral venous plasma.

In contrast to the studies of Dell'Oro and Braun-Menendez are the findings of Peart *et al.* reported in 1961. These investigators

examined the vasoconstricting activity of plasma samples taken from both hypertensive and normotensive rabbits. Upon assay, nearly all samples, whether incubated or unincubated, whether renal or non-renal, wnether from hypertensive or normotensive animals, showed some degree of pressor activity. However, these authors were unable to find any significant difference between the pressor activity of samples obtained from control animals and hypertensive animals, or between the plasmas of renal and non-renal origin. The inability of Peart and his co-workers to demonstrate an increase in the renin content of renal venous plasma taken from hypertensive rabbits was, most likely, due to the use of a renin-angiotensin assay system which was insensitive to the concentration of angiotensin present in the plasma of the normal and hypertensive rabbit (29). Indeed, Lever and Robertson (18) colleagues of Peart, using an improved method of assay for renin (19) were able to detect renin in the blood of normal rabbits. Further, a significant increase in the plasma renin content was observed in hypertensive rabbits.

Recently Skinner, McCubbin and Page (41, 42) have examined the pressor activity of renal venous plasma taken from normotensive and hypertensive dogs. Control samples of plasma from either the normal or hypertensive animals had a basal level of renin which was increased significantly following procedures which reduced mean renal perfusion pressure. In an effort to increase the amount of angiotensin available for assay, the plasma samples were incubated for either one or four hours at 37^{o}C following inhibition of plasma angiotensinase A.

The Vasoconstricting Activity of Lymph

The contradictory results of the studies concerning the presence

or absence of renin in renal vein blood led Peart et al. (28) to postulate that renin, perhaps, leaves the kidney, not by way of the renal vein, but via the renal lymphatics. In 1962 Lever and Peart (17) reported an increased content of renin and angiotensin in renal lymph taken from dogs in which the external diameter of the renal artery was reduced to 2 mm. While renal lymph taken from normal dogs contained angiotensin-like activity, these authors were unable to demonstrate pressor activity in either non-renal lymph or renal venous plasma. Lever and Peart admitted that the difference between the flow rate of renal lymph and renal blood would allow a greater amount of renin to enter the general circulation via the renal venous blood than by way of the lymph. The rate of blood flow may have diluted the renin in the renal venous plasma such that the concentration was below the sensitivity of their assay system.

In 1963 Skinner, McCubbin and Page (40) reported finding angiotensin in the renal venous blood and thoracic duct lymph of dogs following the reduction of the renal artery perfusion pressure to 30-60 mm Hg. Intravenous injection of like volumes of thoracic duct lymph and renal venous plasma into the pentolinium-treated, vagotomized rat indicated that the plasma samples had a slightly greater pressor activity than the thoracic duct lymph samples. However, owing to the much greater flow rate of renal blood than renal lymph these authors concluded that renin is released into the renal venous blood in a much greater amount than appears in the renal lymph following periods of reduced renal perfusion pressure. However, it should be pointed out that the samples of plasma and lymph were not all incubated prior to assay, nor was there any attempt to inhibit angiotensinases present in the plasma and lymph samples.

Hypoxia and Renin Release

Increased renin secretion is associated with partial constriction of the renal artery. Renal artery constriction will reduce renal perfusion pressure and pulse pressure, and it may reduce renal blood flow (37). Reduction in the renal blood flow may produce an hypoxic condition within the renal parenchyma, i.e. the renal blood flow may be inadequate to supply the oxygen requirements of the metabolizing renal tissue. Hypoxia may increase renin secretion if it alters capillary permeability and facilitates movement of renin into the peritubular capillaries (16). However, if the secretion of renin is an active aerobic process, i.e. one requiring oxygen, then hypoxia might decrease renin secretion by the kidney.

In 1942 Huidobro and Braun-Menendez (13) studied the renin secretion of dogs following either intravenous injection of KCN (0.6-1.2 mg/kg) or periods of breathing mixtures of gases low in oxygen (usually 6-8% for 30-80 minutes). No renin was detected in the systemic blood of dogs treated with KCN. Renin was detected in the systemic blood of only two dogs which breathed 6-8% oxygen. In one of these, administration of low oxygen was associated with a fall in blood pressure while the other animals had a low blood pressure throughout the experiment. Further, in both groups of animals subsequent hemorrhage of 4% of the body weight resulted in significant amounts of renin appearing in the systemic circulation. These authors, therefore, concluded that "anoxemia" is not a likely stimulus for renin release.

More recently, Skinner, McCubbin and Page reduced the arterial oxygen saturation of dogs to 50-70% (41). These investigators found no increase in renin release following this maneuver. However, Oliver and

Brody (24) demonstrated an increase in the granularity of the juxtaglomerular cells of rats kept for two weeks in an atmosphere in which the oxygen content was gradually lowered to 7-8%. Since increased granularity of the juxtaglomerular cells has been suggested to be associated with increased renin secretion (12), a decrease in the PO_2 of blood entering the kidney may provide an adequate stimulus for renin release.

Complete occlusion of the renal artery should produce severe hypoxia in the affected kidney. Under these conditions Skinner, et al. (41) did not observe an increased release of renin from the kidney. They suggested that the secretion of renin may depend on an active, aerobic process which cannot function during complete occlusion of the renal artery.

Aim of the Study

A consideration of the findings in the literature just reviewed reveals two questions that are not adequately answered:

1. Is the blood or the lymph the main route of renin release from the kidney?

2. How do local changes in pressure, flow, and oxygen tension affect the output of renin by the kidney?

In an effort to answer these questions, samples of renal venous plasma and hilar lymph were collected before, during and following partial occlusion of the renal artery of anesthetized dogs and assayed for vasoconstrictor activity in the rat. Samples of renal venous plasma were also obtained before, during, and after local changes in renal blood flow and oxygen tension. Assay of these samples was also performed in the rat.

CHAPTER II

EXPERIMENTAL METHODS

Preparation of the Animals

Renal lymph and renal venous plasma were obtained from dogs and assayed in rats for vasoconstrictor activity. The experimental kidney was either naturally perfused or pump perfused. In those experiments in which the kidney was naturally perfused, samples of both renal lymph and renal venous blood were gathered before, during, and following partial occlusion of the renal artery. In those experiments in which the kidney was pump perfused, only samples of renal venous plasma were obtained before and following local changes in oxygen tension and/or blood flow. The natural perfusion experiments will be discussed first, followed by a consideration of the experiments in which an attempt was made to regulate the local blood flow and oxygen tension to the kidney. The preparation of the animal, sampling techniques and vasoconstrictor assay techniques will be discussed in that order.

Natural Perfusion Experiments: Group I

Fifteen mongrel dogs weighing 10-20 kg were used in this study. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). In 10 experiments before proceeding further, a 5 cc sample of blood was withdrawn from the left femoral vein and treated as described later.

Following this, the left kidney was exposed through a flank incision and the peri-renal fat was examined to see if it contained a hilar lymph vessel that might be cannulated. In 10 experiments a hilar renal lymph vessel was cannulated using number 10 polyethylene tubing. After cannulating a lymph vessel or abandoning an unsuccessful attempt, a number 90 polyethylene catheter was threaded into the testicular (or ovarian) vein and positioned to lie in the left renal vein. Left renal artery pressure was measured through a needle catheter placed in the artery and attached to a Statham pressure transducer connected to a Sanborn oscillograph. Following placement of the arterial cannula, each animal was heparinized.

Figure 2 shows a composite of the time course and sample collections that occurred during the natural perfusion experiments (Group I). The specific treatment of all samples collected will be discussed later. Samples of renal venous blood (5 cc each) were obtained through the catheter in the renal vein. After collection of a control renal blood sample and renal lymph (when available), a loop of suture was placed around the left renal artery and tightened until the pressure recorded distal to the loop was lowered from control to the desired value. This decrease in pressure amounted to an average of 35 mm Hg from control levels. The loop was adjusted as necessary to maintain the desired pressure level. A sample of renal venous blood was obtained during the time when the loop was being tightened around the artery. Two samples of renal venous blood and renal lymph (when available) were collected during the constriction period. The average times after pressure reduction to collection of blood and lymph samples were 33 and 64 minutes for the two

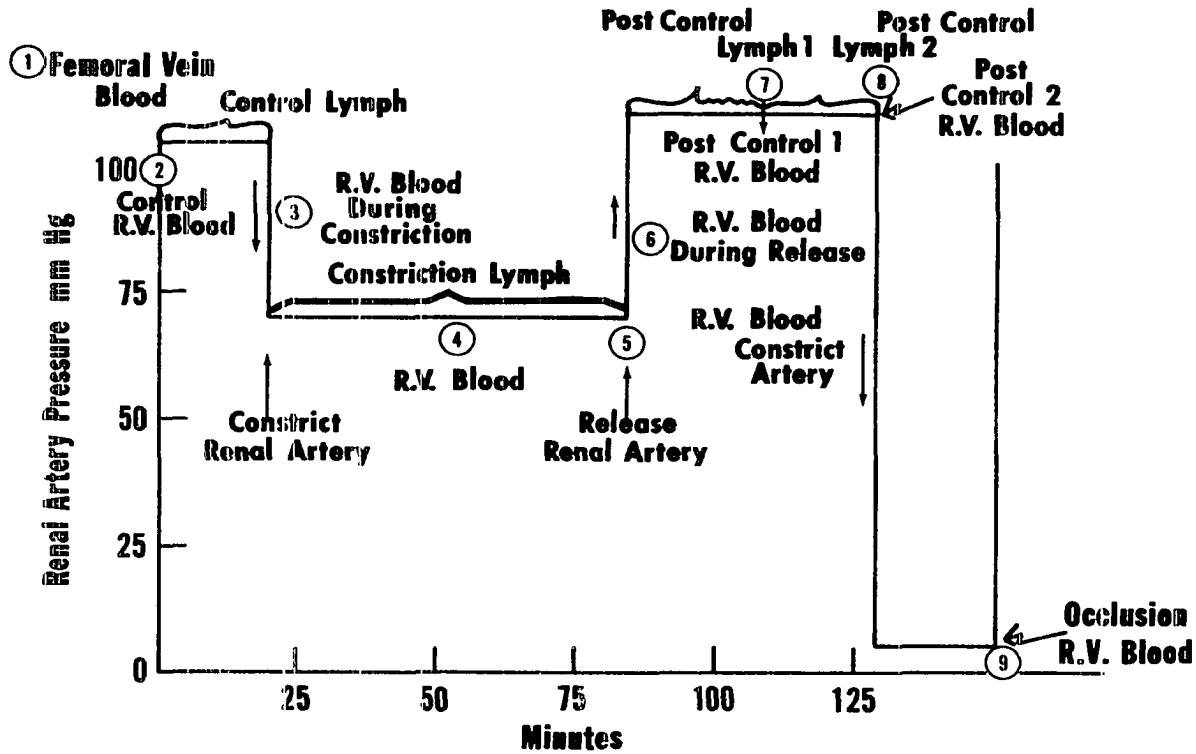

Figure 2. Composite of sample collections from Group I dogs. R. V. = renal vein.

sets of samples. When lymph was being collected, renal vein blood samples were obtained immediately after the collection of each lymph sample. Blood samples were also taken from the renal vein just before the release of the loop and at an average of 24 and 44 minutes following release of the partial occlusion. When possible, two post-control samples of lymph were also gathered at the above times. After obtaining these post-control samples, in 13 dogs, the loop around the renal artery was tightened so as to completely occlude the artery. Following an average of 22 minutes of occlusion, a sample of blood was obtained from the renal vein cannula.

Natural Perfusion Experiments: Group II

Four dogs were studied. They were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and placed on a positive pressure respirator (Harvard Apparatus Co., Model 607). The left kidney was then prepared as described previously for the first group of naturally perfused kidneys. In addition to recording renal arterial pressure, the systemic arterial pressure was monitored throughout the experiment via a cannula placed in the right femoral artery. Lymph samples were obtained in three experiments.

In a manner similar to the Group I animals, the sliding loop of suture was tightened after obtaining control samples of renal lymph and renal venous blood. While in the Group I animals the renal perfusion pressure was reduced on the average to 70 mm Hg on tightening the loop, the renal perfusion pressure in the Group II dogs averaged 59 mm Hg following the constriction. The change in perfusion pressure in this second group of animals, however, averaged 59 mm Hg from control. Renal

venous plasma and renal lymph samples were gathered in a manner similar to the first group of experiments (see Figure 2), except that samples of renal venous plasma were not drawn either during constriction or during release of the renal artery constriction. Samples 1 and 9 shown in Figure 2 were also not drawn from the Group II dogs. Further, an additional sample of blood was taken one minute after release of the constricting loop. Renal venous blood samples were obtained at an average of 28 and 62 minutes after constriction of the artery. One sample of lymph was collected during the period of partial renal artery occlusion. Renal venous blood samples were drawn at 1, 19 and 33 minutes following release of the arterial constriction. Concomitant lymph samples were taken when the second and third post-control blood samples were obtained (as in the manner shown in Figure 2). Specific treatment of the renal lymph and renal venous plasma samples collected from the Group II dogs is described later in this chapter.

The Pump-perfused Kidney

Ten dogs were used in these experiments. They were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and placed on a positive pressure respirator (Harvard Apparatus Co., Model 607). The left kidney was exposed through a flank incision and a number 90 polyethylene cannula placed in the renal vein via the testicular or ovarian vein. The animal was heparinized (4 mg/kg, i.v.). A carotid artery was exposed in the neck of the animal and a needle catheter placed in the artery. Attachment of this catheter to a Statham pressure transducer (model P23Gb) allowed the systemic pressure of the animal to be recorded throughout the experiment on a direct writing oscillograph.

The lungs together with the tracheobronchial tree and accompanying pulmonary artery and vein were then removed intact from a heparinized, anesthetized, donor dog. These lungs were also attached to a positive pressure respirator and a constant flow pump (Sigmamotor pump, model T-6SH, Sigmamotor, Inc., Middleport, N. Y.) was then interposed between the pulmonary vein of the donor lung and the left renal artery of the experimental dog. A second constant flow pump directed the blood from the femoral artery of the experimental dog to the pulmonary artery of the extracorporeal lungs. Figure 3 shows the extracorporeal lung circuit. By altering the oxygen content of the gas mixture ventilating the donor lungs, it was possible to alter, locally, the oxygen tension of the blood entering the experimental kidney. Further, the blood flow to the left kidney could be changed at will by simultaneously increasing or decreasing the speed of the two constant flow pumps which moved the blood through the circuit connecting the left kidney and the extracorporeal lungs.

Renal artery oxygen tension, renal perfusion pressure and the pressure drop across the donor lungs were recorded continuously throughout the experiment. Oxygen tension was measured by an oxygen tension probe (oxygen macro-electrode, Beckman Instruments, Spinco Division, Palo Alto, California) placed in the circuit between the pulmonary vein and the renal artery. The PO_2 measured by the probe was monitored on a gas analyzer (Beckman Instruments, Inc., Model 160) and a simultaneous record was made on a direct-writing oscillograph. Needle catheters placed in the tubing leading to the renal artery and in the tubing leading to the pulmonary artery and vein allowed the monitoring of renal artery perfusion pressure and the pressure drop across the donor lungs.

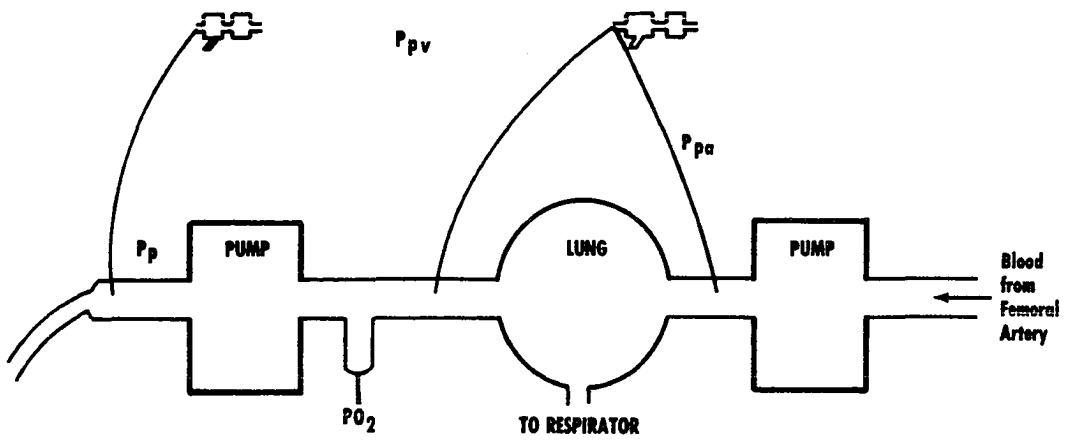

Figure 3. Schematic drawing of the extracorporeal lung circuit (2). P_{pa} = pulmonary artery pressure, P_{pv} = pulmonary vein pressure, P_p = renal perfusion pressure.

Attachment of these catheters to Stathem pressure transducers (Model P23Gb) gave a continuous recording of these pressures on direct-writing oscillographs.

Six animals were anesthetized and prepared as discussed above. The donor lungs were ventilated during the course of each experiment either with a control mixture of 20% O_2-5% CO_2-75% N_2 or with 5% CO_2-95% N_2. The appropriate gas was fed into the intake of the respirator from the corresponding tank of compressed gas. A basketball bladder was interposed between the gas cylinder and the respirator to provide a more even flow of gas. Prior to establishing blood flow between the left kidney and the extracorporeal lungs, ventilation of the donor lungs with the control gaseous mixture (20% O_2-5% CO_2-75% N_2) was begun. The left renal artery was then cannulated with the polyethylene catheter connected to the pulmonary vein of the donor lungs. The circuit, femoral artery-pulmonary artery-lungs-pulmonary vein-left kidney, was then complete, and both the pump directing blood from the femoral artery to the lung and the pump delivering blood to the renal artery were started. The outputs of the pumps were rapidly adjusted so as to attain a steady state in the kidney while maintaining the pressure drop across the extracorporeal circuit within the normal range. Allowing for physiological variations in each experimental kidney, a control rate of renal blood flow was selected which would maintain renal perfusion pressure within the normal range. The output of the pump moving the blood through the renal artery was calibrated directly for all rates except three. These three flow rates were obtained by extrapolation from the calibrated values. The rate and stroke volume of the respirator ventilating the donor lungs was

also adjusted so that the PO_2 probe measured an oxygen tension of approximately 100 mm Hg when the donor lungs were ventilated with the control gas mixture. Ventilation of the donor lung with the control gas mixture (20% O_2-5% CO_2-75% N_2) continued for at least 8 minutes after the renal perfusion pressure and renal, arterial oxygen tension had reached a steady value. Following the control period (period 1) four subsequent experimental periods were carried out in which the renal blood flow and/or renal arterial oxygen tension was altered. Each experimental period was maintained for an average of 13 minutes during which time a new steady-state condition was achieved with respect to the renal perfusion pressure and renal artery oxygen tension. The average changes in renal blood flow, renal blood pressure, and renal artery oxygen tension for each sequential experimental period are presented graphically in a later figure (page 36). In period 2, flow was held constant at the control level, but the donor lungs were then ventilated with 5% CO_2-95% N_2. In period 3 renal arterial blood flow was still held constant at the control level and the lung was ventilated again with the 20% O_2-5% CO_2-75% N_2 control gas mixture; period 3 then was a post-control period. Renal blood flow was severely reduced in period 4 by decreasing the pump outputs. The donor lungs, however, continued to be ventilated with the control gas mixture. In the last experimental period, period 5, flow was continued at the same low level of period 4, but ventilation of the donor lungs was, once again, carried out using 5% CO_2, 95% N_2 gas.

During each experiment, six samples of renal venous blood (5 cc each) were obtained from the renal vein catheter for assay of vasoconstriction activity. A sample was obtained prior to the cannulation of the left renal artery, and again just before the conclusion of each of

the periods described. Treatment of these samples is described in a later section of the experimental methods.

Four additional experiments were conducted using the extracorporeal lung circuit to the experimental kidney. The same preparations and experimental periods were employed. The experimental alterations in renal perfusion pressure and renal artery oxygen tension as well as the experimental sequence are presented graphically later in the text (page 37). Note that after the post control period, the manipulations of periods 4 and 5 in the figure on page 36 were reversed, i.e., the experimental kidney was subjected to a period of combined low blood flow and hypoxia before an experimental period in which ventilation of the donor lungs was returned to the control gas mixture, but the flow was continued at the same low level. Further, in 3 of these 4 experiments, control conditions of the experiment were reinstituted rapidly after the last experimental period by returning to the rate of renal arterial perfusion selected during the control period. A five cc. sample of renal venous blood was drawn for assay purposes 30 seconds after completing the return to control conditions. As in the first series of six animals, 5 cc samples of renal venous blood were previously obtained before instituting controlled flow to the experimental kidney and also just before the termination of each experimental period.

Treatment of Samples

The samples of renal venous blood and renal lymph collected during the experiments described above were subjected to various treatments before being assayed for vasopressor activity by intravenous injection into a suitably prepared rat. The treatment given the blood and lymph

samples obtained in those experiments in which the kidney was naturally perfused is represented schematically in Figure 4. Also represented in Figure 4 is the treatment afforded those samples of renal venous blood gathered when the kidney was pump-perfused using the extracorporeal lung circuit.

In the first group of 15 animals in which the left kidney was naturally perfused through its renal artery, each 5 cc sample of blood drawn was immediately placed into a tube containing 0.5 cc of 5 x 10^{-3} M calcium disodium versenate and centrifuged in a model HR-I International refrigerated centrifuge (chamber temperature at 4°C) at 5927 G for 7 minutes. After centrifuging, the supernatant plasma was divided into two portions, placed in separate polyethylene tubes and frozen in a dry ice-alcohol bath. Fifteen to twenty minutes elapsed from the time of drawing a sample until it was frozen. Twenty-four to forty-eight hours after the collection of the samples, one set of aliquots was incubated at 37°C for 1 hour, the other set for 4 hours at 37°C. At the conclusion of the incubation period, the samples were again frozen in the dry-ice alcohol bath.

The treatment of the renal venous blood samples drawn from the second group of experimental animals in which the kidney was naturally perfused differed from the first group. In these experiments the blood samples were immediately placed into a tube containing 0.5 cc of 18 x 10^{-3} M EDTA (disodium salt) and placed in an ice bath until the collection of all samples was completed. These samples were then centrifuged in the same manner as the Group I samples. After centrifugation the supernatant plasma was divided into two equal portions one of which was frozen, while the other aliquot was incubated at 37°C for 4 hours and then frozen.

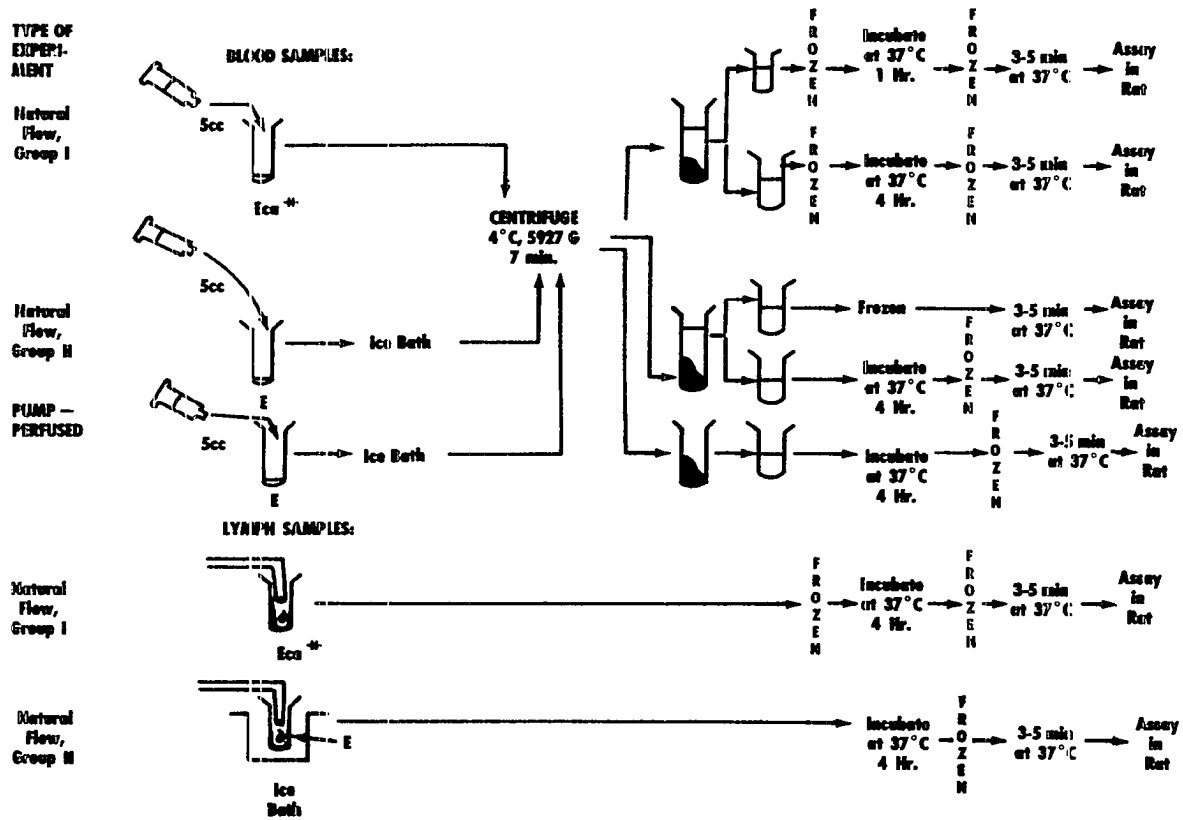

Figure 4. Treatment of plasma and lymph for vasopressor assay. E_{Ca}^{++} = calcium-complexed EDTA. E = uncomplexed EDTA.

The blood samples from the pump-perfused kidneys were treated like the blood samples obtained during the second group of natural perfusion experiments. However, no unincubated plasma samples were prepared for assay.

Samples of renal lymph were obtained from both the Group I and Group II experiments in which the kidney was naturally perfused. However, the treatment given the lymph samples of Group I and Group II animals differed. Lymph from the first group was collected at room temperature in glass tubes in which 0.2 cc of 5×10^{-3} M calcium disodium versenate had been taken to dryness. After collection, the samples were frozen. Twenty-four to forty-eight hours later, these samples were incubated at 37°C for 4 hours and then refrozen. Lymph from the second group of dogs was collected at 4°C in glass tubes in which 0.2 cc of 3×10^{-3} M EDTA (disodium salt) had been taken to dryness. After all the lymph samples in a given experiment were collected, they were placed in a water bath at 37°C for 4 hours. Following incubation they were frozen until assay in the rat.

The incubation of the various plasma and lymph samples was carried out to allow for maximum formation of angiotensin II as shown in Figure 1. Note also in Figure 1 that angiotensinases in plasma will act to destroy angiotensin II. Studies of the aminopeptidase, angiotensinase A, have shown it to be calcium activated (15, 23). EDTA (disodium salt) can form a stable complex with calcium. Thus, if EDTA is added to plasma it will complex Ca^{++} and inactivate the angiotensinase A which would otherwise attack angiotensin II. The EDTA added to the blood and lymph samples from the first group of naturally perfused kidneys was already

complexed with calcium and, therefore, could not function to remove Ca^{++} from the plasma or lymph. However, samples of plasma and lymph from the Group II natural perfusion experiments were collected using uncomplexed EDTA capable of removing ionized calcium from the samples. All samples of blood from the pump-perfused kidneys were likewise collected into uncomplexed EDTA.

Assay of Samples

The samples of renal lymph and renal venous plasma were assayed after the method of Skinner, McCubbin, and Page (41). The rats used in the assay of the samples obtained from the Group I, natural perfusion experiments were male and female King-Holtzman rats weighing 145-420 gms. All other samples were assayed in female Holtzman rats weighing 150-250 grams. Each rat was anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and given 0.4 cc (4 mg) pentolinium tartrate subcutaneously. Pentolinium tartrate is a ganglionic blocking agent which prevents depolarization of the postganglionic membrane (48). In our preparation pentolinium tartrate produced a stable preparation with a controlled hypotension, a condition advantageous for the assay of a vasoconstrictor substance.

After surgical anesthesia was achieved, the trachea was cannulated, and a bilateral vagotomy performed. The right iliac vein was then cannulated using a short number 10 polyethylene cannula. The volume of the cannula was such that less than 0.05 cc of solution would fill it. Following cannulation of the vein, the animal was heparinized. The systemic arterial pressure in all rats was monitored through an arterial cannula which was inserted into the right carotid artery and attached to

a Statham pressure transducer. Following stabilization of the arterial pressure, the reactivity of the preparation was tested by the injection of 2 nanograms (nanogram = 10^{-9} grams) of synthetic angiotensin amide. Each injection of test substance was preceeded by an injection of 0.05 cc of the material to be tested in order to clear the venous catheter of any previously injected material. The test volume (that volume injected following the flush of the catheter) for all lymph samples was 0.1 cc, while the test volumes for plasma samples varied according to the size of the assay animal.

The plasma and lymph samples to be assayed were kept frozen until just before assay at which time each sample was warmed by placing it for 3-5 minutes in a water bath at 37°C.

CHAPTER III

RESULTS

Natural Perfusion Experiments

The pressor activity of plasma samples obtained from dogs in which the kidney was naturally perfused is shown in Figures 5 and 6. Figure 5 shows the response of the assay animal after injection of plasma samples treated with calcium-complexed EDTA and incubated either one or four hours at 37°C. The data was obtained from experiments on 15 dogs. Figure 6 represents the pressor response produced by the intravenous injection of plasma samples treated with uncomplexed EDTA and assayed either without prior incubation at 37°C or following four hours of incubation at 37°C. Data from experiments on 4 dogs is represented. Both Figure 5 and Figure 6 indicate the average of the maximum increase in the diastolic pressure of the assay rat following intravenous injection of plasma samples. Plasma samples are listed on the abscissa. The increase in pressure obtained from injection of corresponding plasma samples are superimposed on one another in both Figure 5 and Figure 6. For example, the injection of the control plasma (C in Figure 5) elevated the diastolic pressure of the test rats an average of 6 mm Hg, if the samples had been incubated for one hour. Injection of the corresponding control plasmas incubated for four hours produced an average increase of 9.4 mm Hg in the diastolic pressure of the test animals on assay. Each

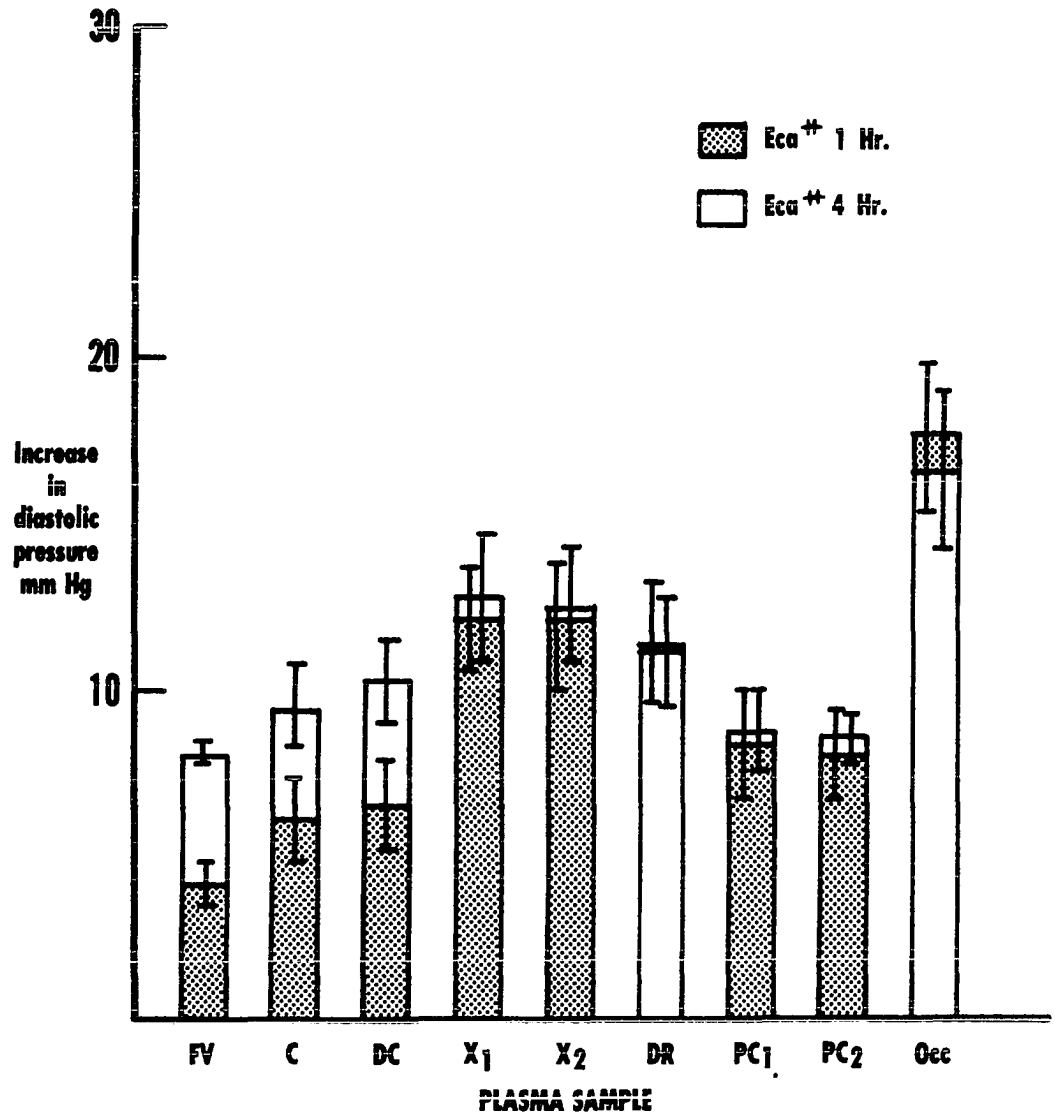

Figure 5. Assay of plasma from Group I dogs. E_{Ca}^{++} = treated with calcium-complexed EDTA and incubated one hour, E_{Ca}^{++} = treated with calcium-complexed EDTA and incubated 4 hours. FV = femoral venous plasma, C = control plasma, DC = plasma, during arterial constriction, X_1 = constriction 1 plasma, X_2 = constriction 2 plasma, DR = plasma, during arterial release, PC_1 = post-control 1 plasma, PC_2 = post-control 2 plasma, Occ = complete occlusion plasma. Injection volumes = 0.1 cc-0.3 cc per test per sample.

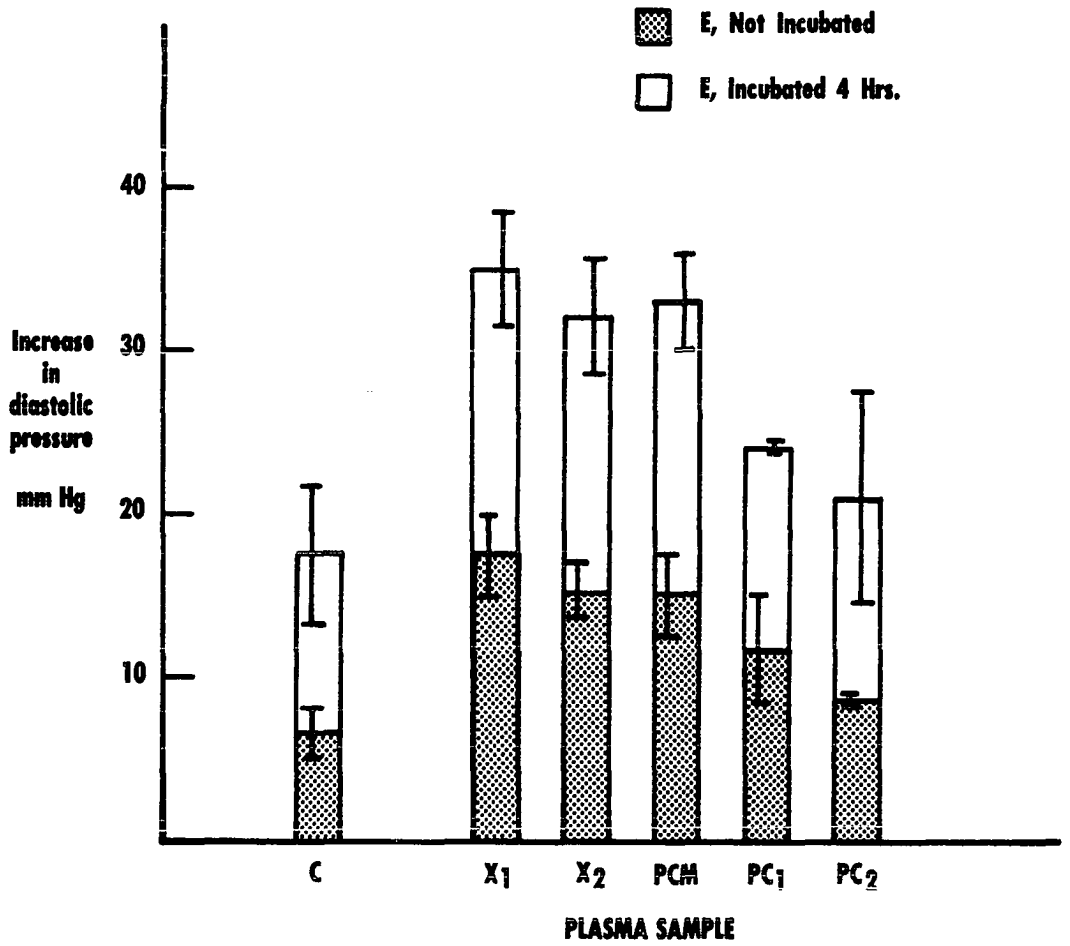

Figure 6. Assay of plasma from Group II dogs. ▨ E = treated with uncomplexed EDTA and unincubated, □ E = treated with uncomplexed EDTA and incubated 4 hours. C = control plasma, X_1 = constriction 1 plasma, X_2 = constriction 2 plasma, PCM = plasma taken 1 minute after release, PC_1 = post-control 1 plasma, PC_2 = post-control 2 plasma. Injection volume = 0.2 cc per test per sample.

response, therefore, is depicted as a change from a control level (0, on the graph) to whatever level is indicated. The standard error of each mean response is indicated by the I bar about each mean. The letters on the abscissa below each bar represent the particular plasma samples that gave the response shown above it. The assay of femoral venous plasma (FV), control plasma (C), plasma taken during the act of constricting the renal artery (DC), plasma taken 33 minutes after arterial constriction (X_1) and plasma drawn 64 minutes after constriction (X_2) is represented in Figure 5. Samples of plasma taken during release of the constriction (DR), as well as 24 minutes (PC_1) and 44 minutes (PC_2) after release of the partial occlusion were also assayed, and the results are graphed in Figure 5. Finally, the vascular response of the rat to the injection of plasma obtained after 22 minutes of complete occlusion appears in Figure 5 also. The symbols used to represent the various plasma samples shown in Figure 6 have the same designations as the corresponding samples in Figure 5. The results of the assay of plasma samples taken an average of 28 minutes (X_1) and 62 minutes after partial constriction of the artery are shown. Likewise, the pressor effect of the injection of two post-control samples of plasma drawn an average of 19 minutes (PC_1) and 33 minutes (PC_2) after release of the constricting loop is represented. The pressor effect of injection of a plasma sample obtained 1 minute after release of the partial occlusion (sample PCM) appears also.

The results obtained on the bioassay of the plasma samples treated with calcium-complexed EDTA and incubated either one or four hours (Figure 5) will be considered first. Following this, the vasopressor

activity of plasma samples treated with uncomplexed EDTA and assayed either without prior incubation or after four hours of incubation (Figure 6) will be examined. The samples which comprise Figure 5 are the Group I samples, while those samples depicted in Figure 6 are Group II samples.

Vasopressor Activity of Plasma from Group I Dogs: Figure 5

The plasmas incubated for one hour will be discussed first. Samples of renal venous blood drawn at 33 and 64 minutes following reduction of the renal artery pressure (samples X_1 and X_2) show constrictor activity. The response obtained with these samples, i.e. X_1 and X_2, appear greater than either control or post-control samples. Blood obtained following twenty-two minutes of complete occlusion shows a greater pressor activity than either control plasma, previous constriction samples, or post-control renal venous plasma. Note also the slight constrictor activity found in plasmas from femoral venous, control renal venous, and renal venous blood obtained during constriction of the renal artery.

The vasopressor activity of the plasmas incubated four hours exhibits a pattern (from control plasma to occlusion plasma) similar to that of the corresponding plasmas incubated for one hour. However, only the sample obtained following complete arterial occlusion appears to be different than the control sample. Note also that samples incubated for four hours are not different than the samples incubated for one hour except, perhaps, in the case of blood samples obtained from the femoral vein, during the control period, and during the constriction of the renal artery.

Vasopressor Activity of Plasma from Group II Dogs: Figure 6

Assay of plasma samples that were not incubated at 37°C prior to assay indicates that samples drawn at 28 and 62 minutes following partial occlusion of the renal artery show a greater constrictor activity than the control plasma. Further, the sample of plasma taken one minute after release of the occlusion exhibits a pressor activity similar to the two samples of plasma taken during the period of renal artery constriction. The two post-control samples of plasma do not appear to have a pressor activity much different from the pressor activity of the control plasma. The corresponding samples of plasma obtained at the same time in the experiment, but incubated for four hours, exhibit a pattern of changing pressor activity (from the control to post-control sample) similar to the unincubated plasma samples. Again, the samples of plasma obtained during constriction and one minute following release of the constriction appear different than the control sample. The post-control samples indicate a gradual return to the control levels of plasma pressor activity in the 33 minutes following release of the arterial occlusion. However, the incubated and unincubated sets of plasma are quantitatively quite different. In each case the sample incubated four hours caused over twice the average pressor response in the test animal as did the corresponding unincubated sample.

Vasopressor Activity of the Lymph Samples

Figure 7 represents the results obtained on the biological assay of samples of hilar renal lymph gathered during the Group I and Group II natural flow experiments. The Group I lymph samples were treated with calcium-complexed EDTA and incubated for four hours. The Group II lymph

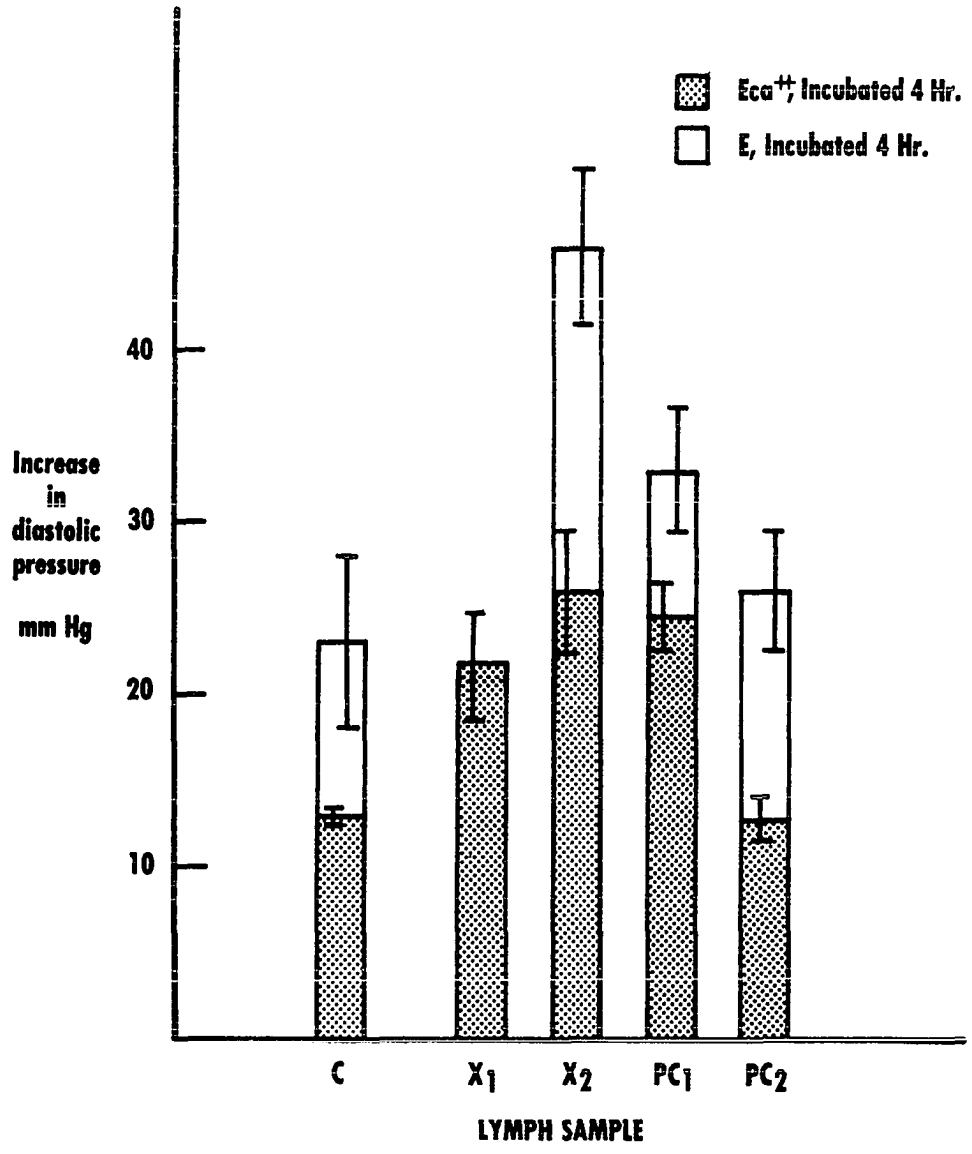

Figure 7. Assay of lymph from Group I and II dogs. E_{Ca}^{++} = treated with calcium-complexed EDTA and incubated 4 hours. E = treated with uncomplexed EDTA and incubated 4 hours. C = control lymph, X_1 = constriction lymph 1, X_2 = constriction lymph 2, PC_1 = post-control lymph 1, PC_2 = post-control lymph 2. Injection volume 0.1 cc per test per sample.

samples were treated with calcium-free EDTA and incubated for four hours also. The individual lymph samples assayed are indicated on the abscissa. The lettered designations stand for control lymph (C), constriction lymph (X_1 and X_2) and post-control lymph (PC_1 and PC_2). The graph is read similar to Figures 5 and 6. The results from the two sets of samples are superimposed on one another, and each response is read as a given change from the zero or control level. The Group I lymph data was obtained from 10 dogs, while the Group II lymph data was obtained from 3 dogs. Only one sample of constriction lymph was assayed from each of the Group II dogs, while in 7 of the Group I dogs, two consecutive samples of lymph (X_1 and X_2) were obtained during the period of partial arterial occlusion.

Of the lymph samples treated with calcium-complexed EDTA, the samples of lymph taken 33 and 65 minutes after constriction of the artery, as well as the first post-control sample collected for 27 minutes following release of the occlusion, appears to have a greater pressor activity than either the control lymph sample or the second post-control lymph sample. Indeed, similar vasopressor activity is present in the two samples of lymph obtained while the renal artery was constricted and also in the first sample of lymph collected after release of the constriction. The samples of renal lymph treated with calcium-free EDTA exhibit a pattern of changing pressor activity similar to that obtained on assay of the samples treated with calcium-complexed EDTA. However, only the constriction lymph sample appears very different from either the control or post-control lymph samples. Unlike the Group I lymph samples, the first post-control lymph collected for 20 minutes following

release of the occlusion appears to have less vasopressor activity than the samples obtained after 64 minutes of partial occlusion of the renal artery. Further, the pressor response elicited by the Group II samples in each case appears greater than the corresponding pressor response produced in the test animal by injection of the Group I samples.

The volume of lymph assayed was in all but one case less than the corresponding plasma samples. However, the lymph samples from both the Group I and Group II show a greater vasopressor activity than any of the corresponding plasma samples.

Pump-perfused Kidneys

The results of the biological assay of plasma samples obtained during pump-perfusion of the kidney using the extracorporeal lung circuit are shown in Figures 8 and 9. The top half of each figure shows the value recorded for renal perfusion pressure, renal artery oxygen tension, and renal blood flow of the experimental kidney at the time of the drawing of each of the samples of renal venous blood. The operating conditions of each period are indicated by the letter designations above that period. These conditions are: (1) control conditions after the perfusion pumps start (CAP), (2) ventilation of the donor lungs with 5% CO_2-95% N_2 gas, (3) a post-control condition (PC) i.e. a return to ventilation of the extracorporeal lungs with the 20% O_2-5% CO_2-75% N_2 mixture, (4) a period of controlled low flow (LF), and, (5) a period of combined low flow and hypoxia (LF + 5% CO_2-95% N_2). In both Figure 8 and Figure 9, a control and a post-control period (periods 1 and 3) bracket a period in which the extracorporeal lung circuit is ventilated with 5% CO_2-95% N_2. However, the order of periods 4 and 5 in Figure 8 is

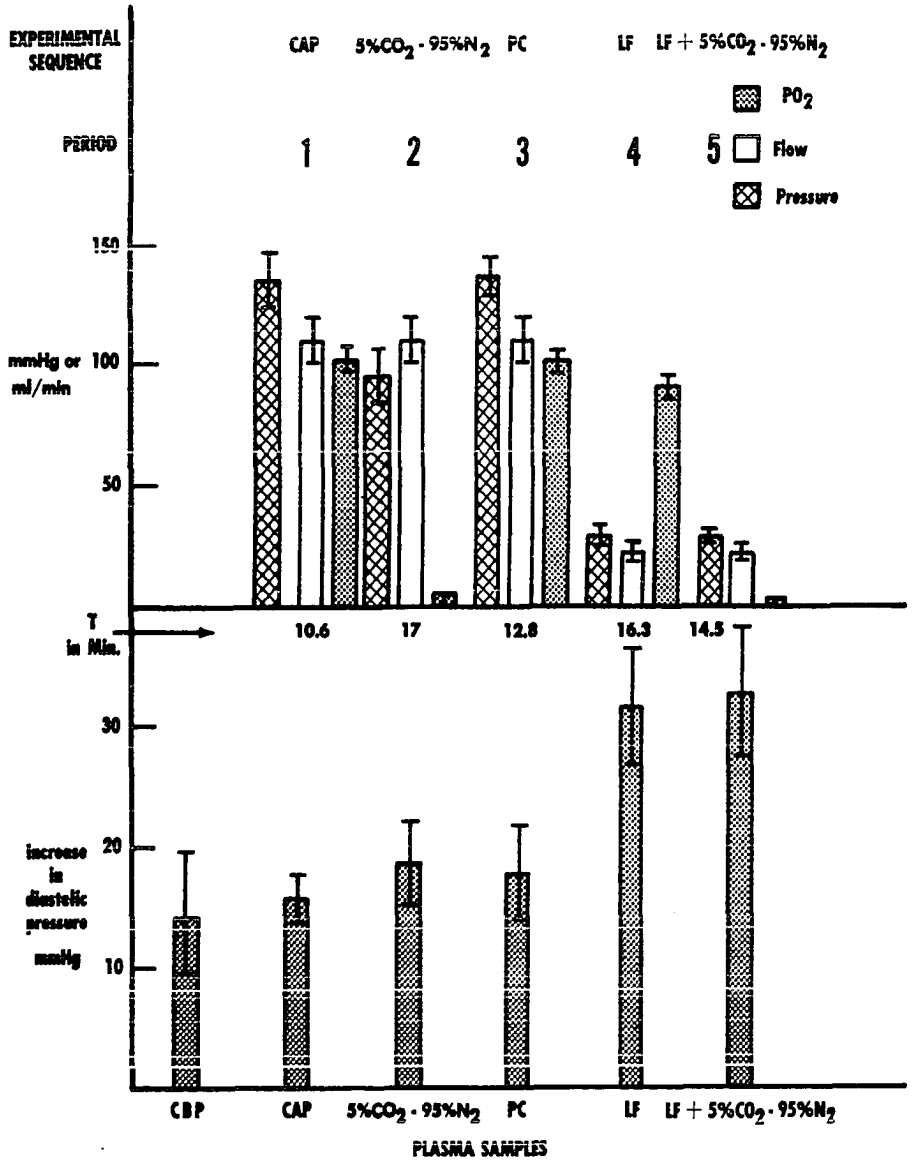

Figure 8. Assay of plasma samples from pump-perfused kidneys: Sequence I Top: Changes in oxygen tension ( ), blood flow ( ), and perfusion pressure ( ) in the dog with the following sequence: control before pump (CBP), control after pump (CAP), ventilation with 5% CO_2-95% N_2), post-control (PC), low flow (LF), and low flow and hypoxia (LF + 5% CO_2-95% N_2). T = time after institution of the experimental procedure at which the plasma sample was drawn. Bottom: Bioassay of the renal venous plasma samples obtained at time (T) in each period.

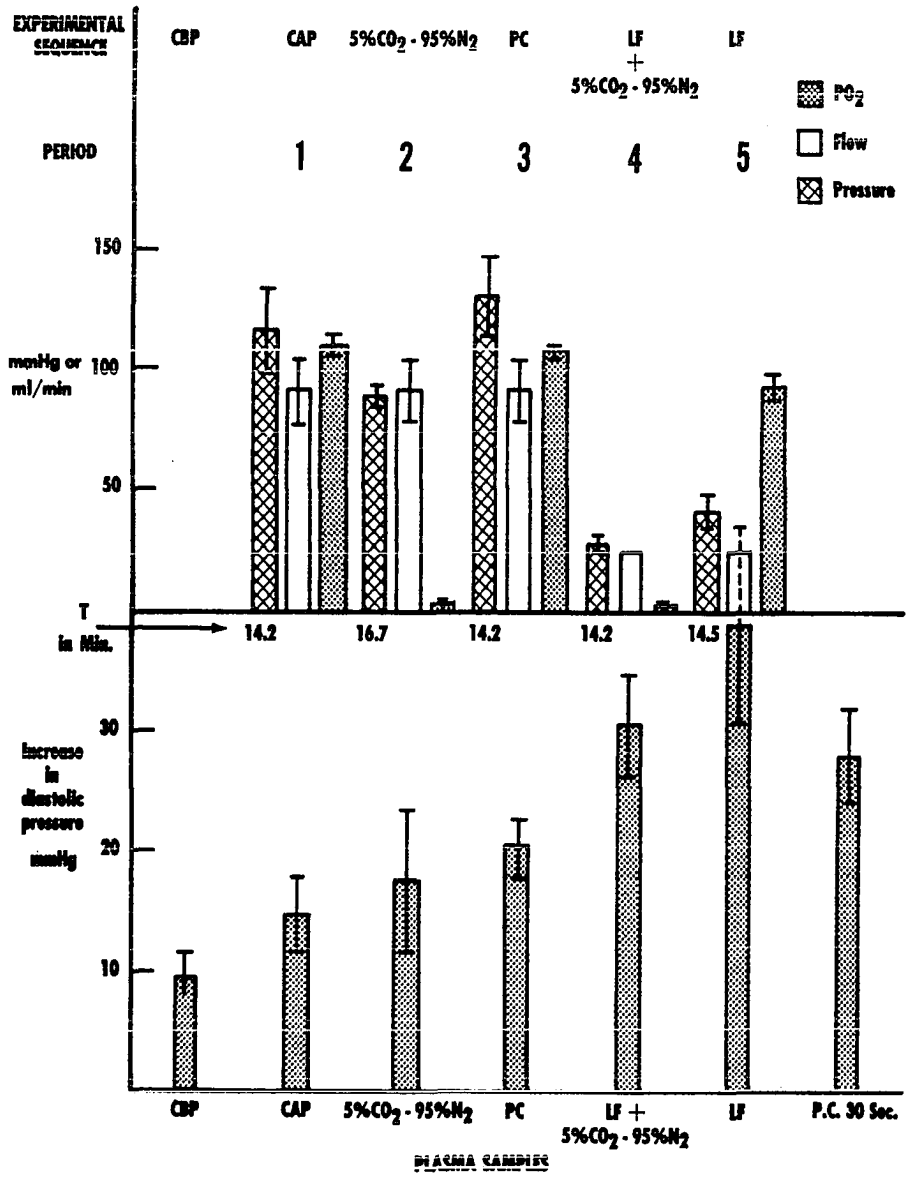

Figure 9. Assay of samples from pump-perfused kidneys; Sequence II Top: Changes in oxygen tension ( ), blood flow ( ), and perfusion pressure ( ) in the dog with the following sequence. Control before pump (CBP), control after pump (CAP), ventilation with 5% CO_2-95% N_2, post-control (PC), low flow (LF) + 5% CO_2-95% N_2, and low flow (LF). T = time after institution of the experimental procedure at which the sample was drawn. Bottom: Bioassay of the renal venous plasma samples obtained at time (T) in each period. P.C.-30 Sec = post-control plasma taken 30 seconds after the end of period 5.

reversed in the experiments represented in Figure 9. The lower half of both these figures present the results of the bioassay of plasma samples drawn at time T (in minutes) after the institution of the specific experimental conditions designated below each bar. These conditions correspond with those shown in the upper half of the figure. One additional sample, a control sample taken before pump-perfusion of the kidney began (CBP) is shown assayed. Figure 8 represents data obtained from 6 dogs while Figure 9 shows data gathered from 4 dogs. All the data represented are average values with the standard error of the mean indicated by the I on each bar of the graphs.

Figure 8 indicates that after ventilation of the donor lungs with a mixture of 5% CO_2-95% N_2 for 17 minutes, the renal perfusion pressure decreased an average of 40 mm Hg, although renal blood flow was held constant at 110 ml/min. The hypoxia developed was severe; the oxygen tension of the perfusing blood was only 7 mm Hg. The bioassay of the plasma samples for vasoconstrictor activity indicate that renal venous blood drawn during a period of severe hypoxia exhibited apparently no greater pressor activity than any of the three control samples (CBP, CAP or PC). However, comparing the vasopressor activity of the post-control plasma (PC) with either the plasma taken after 16 minutes of low flow (22 ml/min) or following 14.5 minutes of combined hypoxia and low flow, it appears that both these samples have a greater pressor activity than any of the samples from the preceding experimental periods. Note also that when renal blood flow was reduced to 22 ml/min, renal perfusion pressure fell to 29 mm Hg from a post-control value of 136 mm Hg. Concurrently, the oxygen tension of the perfusing blood fell from a

post-control level of 101 mm Hg to 91 mm Hg at 16 minutes after reducing the renal blood flow. Subsequent ventilation of the donor lungs with the anoxic gas mixture dropped the PO_2 of the renal artery blood to 2 mm Hg. At the same time, the hypoxia produced a negligible change in renal perfusion pressure.

The results shown in Figure 9 are similar to those presented in Figure 8. After 17 minutes of ventilation of the extracorporeal lungs with a gas mixture devoid of oxygen, the oxygen tension of the blood entering the experimental kidney fell to 3 mm Hg from a control level of 110 mm Hg. At the same time the renal perfusion pressure fell from 116 mm Hg to 88 mm Hg. Again, flow was held constant throughout the first three experimental periods. Return to ventilation of the donor lungs with the control mixture (20% O_2-5% CO_2-75% N_2) restored the renal artery oxygen tension to control level, but the post-control renal perfusion pressure was increased 15 mm Hg above the control value. Reduction of the renal blood flow to 34 ml/min from a control value of 90 ml/min resulted in a concomitant decrease in renal perfusion pressure to 27 mm Hg at a time when the oxygen tension of the perfusing blood was reduced to 2 mm Hg by ventilation of the extracorporeal lungs with 5% CO_2-95% N_2. Return to ventilation with control mixture at continued low flow resulted in an increase of the PO_2 to 94 mm Hg, 14 mm Hg less than the post-control level. As with the measured renal parameters, the results of the bioassay of the plasma samples represented in Figure 9 are similar to the results graphed in Figure 8. The pressor activity of plasma obtained 17 minutes after exposure of the donor lungs to an anoxic gas mixture did not differ from the pressor activity of any of

the control plasmas. However, (as shown in Figure 9) the plasma obtained during the post-control period appears to have greater vasoconstrictor activity than control plasma drawn prior to the beginning of pump-perfusion of the experimental kidney (sample CBP). Further, both samples of plasma obtained after the renal blood flow had been reduced to 24 ml/min have a greater pressor activity than any of the samples obtained earlier in the experimental sequence.

The final sample assayed in the sequence shown in Figure 9 (sample P.C.-30 Sec) represents the pressor activity found in renal venous blood samples taken 30 seconds after the renal blood flow had been returned to the control level following the conclusion of the 5th experimental period. The pressor activity of this plasma appears to be no different than the vasoconstricting activity found in plasma during periods 4 and 5. However, the pressor activity of the P.C.-30 Sec sample may be only slightly different than that of the post-control plasma obtained during period 3.

Summary of Results

The results of this study indicate that a vasopressor substance is present in renal hilar lymph and renal venous plasma. Reducing the perfusion pressure to the experimental kidney may result in an increased vasopressor activity appearing in both the renal lymph and renal venous plasma. Severe reduction of the oxygen tension of the blood perfusing the experimental kidney has little effect on the secretion of the vasopressor substance into the renal venous effluent.

CHAPTER IV

DISCUSSION

The results of the present study indicate that the pressor substance assayed was likely angiotensin II generated by the constituents of the renin-angiotensin system present in both renal venous plasma and renal lymph. Throughout the discussion an attempt will be made to relate the findings of this study to the operation of the renin-angiotensin system. Consideration will also be given to the various mechanisms which may function to control renin secretion.

Natural Perfusion Experiments

With the exception of plasma samples which were treated with calcium-complexed EDTA and incubated four hours, the plasma samples obtained while the renal artery was constricted (samples X_1 and X_2, Figures 5 and 6) show a greater pressor activity than control samples drawn prior to the constriction of the renal artery. Release of the constricting loop led to a gradual return of the pressor activity of the plasma to control levels. This return to control pressor levels is evident in plasma samples obtained at an average of either 24 minutes (sample PC_1, Figure 5) or 33 minutes (sample PC_2, Figure 6) after release of the arterial occlusion. These findings are in agreement with those of Skinner, McCubbin and Page (40) who reported increased pressor activity

appearing in renal venous blood after reduction of the renal artery perfusion pressure to levels between 30 and 60 mm Hg. Vasopressor activity disappeared from the renal venous plasma within 30 minutes after reinstituting normal perfusion of the kidney.

Other experiments by Skinner, McCubbin and Page (41, 42) indicated that the pressor activity of a given plasma sample could be increased by pretreatment of the samples with EDTA (disodium salt) coupled with incubation of the samples for either 1 or 4 hours at 37°C. This increased pressor activity of incubated, EDTA treated plasma is attributable to two factors: the enzymatic nature of renin and the inactivation of the angiotensinase A of the plasma. Since the renin-angiotensin system is an enzyme system, incubation of the reaction mixture at 37°C may be expected to increase the rate of formation of angiotensin (49). Therefore, in the presence of excess substrate, the longer the reaction mixture is incubated, the greater will be the amount of the product, i.e. angiotensin, produced (31). The destruction of the angiotensin II formed in the incubation mixture is retarded by addition of disodium-EDTA to the mixture prior to incubation (15, 23). The EDTA complexes ionized calcium, and, thus, serves to inactivate angiotensinase A which requires ionized calcium in order to act on angiotensin II.

The action of plasma angiotensinases may serve to explain the pattern of pressor activity shown in Figure 5. Recall that Figure 5 represents the results of the bioassay of two sets of aliquots of plasma obtained during the natural perfusion experiments. One set of aliquots was incubated for one hour; the other set was incubated for 4 hours. If angiotensin II was generated during the incubation of the samples, one

might expect that each sample incubated for four hours would have a greater pressor activity than the corresponding sample incubated for only one hour (41, 42). However, only the femoral venous plasma, the control plasma, and plasma obtained during constriction show a greater pressor response in samples incubated for the longer period of time. All other plasma samples incubated for 4 hours contain no greater pressor activity than the corresponding samples incubated for only one hour. All the plasma samples represented in Figure 5 were treated with calcium-complexed EDTA. In other words, the incubation mixtures did not contain an agent which would inhibit angiotensinases present in the plasma. Further, at normal plasma substrate concentration, the rate of formation of angiotensin is dependent only on the renin concentration, while the rate of destruction of angiotensin is proportional to the concentration of angiotensin and angiotensinases present (31, 33). Thus, in the femoral vein, control, and during constriction samples, it might be that the rate of formation of angiotensin is greater than the rate of destruction, and the longer incubation period results in a greater net formation of a constrictor. Constriction of the renal artery may release more renin, producing more constrictor, but the increased incubation time may increase the concentration of angiotensinase substrate such that the net concentration of angiotensin available for bioassay is no longer different than that concentration of constrictor produced during a one hour incubation period.

The fact that there was no apparent difference in the two sets of plasma samples obtained any time after constriction of the renal artery might also be due to the amount of pressor substance present in

these samples. Perhaps the amount of pressor substance produced after one hour of incubation was sufficient to produce a maximal response on injection into the test animal. If this were so, continued incubation might produce yet a greater concentration of constrictor but this increased concentration would not be detected in a test animal which responded maximally to a lesser concentration of angiotensin. However, this idea seems untenable because the sample of plasma obtained with complete occlusion of the renal artery and incubated one hour produced a greater pressor response in the assay rat than did the plasmas gathered during the period of renal artery constriction. Thus, it appears likely that the pattern of pressor activities presented by the plasma samples shown in Figure 5 is due, in part, to the presence of active angiotensinases.

Figure 6 represents the results obtained on assay of samples of plasma to which uncomplexed EDTA was added to inhibit the action of the angiotensinase of the plasma. In each case the activity of samples incubated for four hours was greater than the pressor effect of the corresponding unincubated plasma samples. The appearance of increased pressor activity in renal vein blood following incubation suggests that the renin-angiotensin system is operating within the samples (41). Further, the shape of the pressure curve elicited in the rat on assay of the unknown samples was the same as that produced on injection of a preparation of purified angiotensin amide. This finding also supports the view that the pressor substance assayed and angiotensin have identity (40, 41). Another indication that the vasopressor substance assayed in our experiments was angiotensin II arises from the fact that increased

pressor activity appeared in the renal venous effluent following constriction of the renal artery. This increased pressor activity can be regarded as indicating an increased secretion of renin (18). However, Selkurt, et al. (38) reported the reduction of renal plasma flow when renal perfusion pressure to the canine kidney was reduced to 73 mm Hg. A further reduction of the renal perfusion pressure to 50 mm Hg caused a marked drop in the renal plasma flow as well as in the glomerular filtration rate. Reinstitution of normal perfusion to the kidney caused the renal plasma flow of the experimental kidney to return to 81% of the control value. In the natural perfusion experiments of this present study, the renal perfusion pressure during the period of arterial constriction averaged either 70 mm Hg (Group I dogs) or 59 mm Hg (Group II dogs). Therefore, it is reasonable to assume that the renal plasma flow through the experimental kidney was much reduced following constriction of the renal artery. The increased pressor activity found in plasma samples obtained a given time after renal artery constriction might be explained by a concentration effect; during the period of constriction the rate of release of renin into the renal vein blood may have remained constant, while the renal plasma flow through the kidney decreased. If this were the case, the renin content of the renal venous effluent would increase in the absence of an increase in the rate of renin secretion. However, the results of the natural perfusion experiments indicate that the increased pressor activity seen in the plasma samples obtained during the period of renal artery constriction cannot be explained on the basis of reduced blood flow alone. The pressor activity of plasma obtained one minute after release of the constriction of the renal artery (sample PCM, Figure 6) had a pressor activity almost 100% greater than

did the control sample and this at a time when the renal plasma flow might have been only 81% of control (38). Also, the plasma drawn during release of the renal artery (sample DR, Figure 5) and incubated for one hour had a pressor activity almost 80% greater than the corresponding control plasma. Thus, it appears reasonable that the reduction of the renal perfusion pressure is followed by an increased secretion of renin into the renal venous blood. This increased renin secretion is evidenced by a greater vasopressor activity in plasma samples obtained after constriction of the renal artery as compared with the control plasma. This increased vasopressor activity results from the production of angiotensin II in a reaction mixture designed to increase the production as well as the biological half-life of angiotensin II. It should be stated, however, that the results of this study offer no means to ascertain that the pressor substance assayed was, in fact, angiotensin II. The samples of hilar renal lymph collected during the natural perfusion experiments exhibit a pattern of pressor activity similar to the corresponding plasma samples. Constriction of the renal artery is followed by an increased pressor activity appearing in renal lymph. Release of the constricting ligature is followed by a gradual decrease in the constrictor properties of the lymph to control levels. The results of these experiments agree, at least in part, with similar studies (18, 40) which have shown that a renin or angiotensin-like substance is present in lymph. Although Lever and Peart (18) in 1962 reported finding renin and angiotensin-like activity in renal lymph which activity increased on stenosis of the renal artery, these investigations were unable to detect vasopressor activity in the renal vein blood of animals which contributed

pressor lymph. Skinner, McCubbin and Page (40) on the other hand, found vasopressor activity in both renal venous blood and thoracic duct lymph. However, these authors consistantly found more angiotensin per unit volume of renal venous plasma than per unit volume of thoracic duct lymph. Renal hilar lymph, not thoracic duct lymph, was assayed in the present study. In all cases the pressor activity of the hilar renal lymph per unit volume assayed was greater than the corresponding plasma samples. This was true even in the case of the lymph samples which were treated with calcium-complexed EDTA. However, the rate of renal blood flow is much greater than the rate of renal lymph flow. Therefore, while the amount of vasopressor agent per unit volume of lymph is greater than that per unit volume of renal venous blood, the absolute amount of constrictor leaving the kidney per unit time is likely greater by way of the renal venous blood than via the renal lymph. Unlike the corresponding plasma samples, lymph samples obtained after constriction of the renal artery and treated with calcium-complexed EDTA show a greater pressor activity than either the control or second post-control lymph sample. This difference in activity between the plasma and lymph samples taken during the Group I natural perfusion experiments is, perhaps, best explained by a consideration of the composition of lymph. Friedman, Marx and Lindner (6) reported that the renin substrate level was similar for both plasma and lymph. The angiotensinase concentration of lymph, on the other hand, was considerably less than that of plasma. Thus, the increased pressor activity present in lymph samples treated with calcium-complexed EDTA might be a consequence of the angiotensinase content of lymph. The higher concentration of angiotensinase in the plasma may

inactivate a greater amount of angiotensin per unit time than does the lesser quantity of angiotensinase in the lymph. Reduction of the renal artery perfusion pressure from 110 mm Hg to 60 mm Hg decreases the hilar lymph flow rate about fifty per cent (11). Thus, the increased vasopressor activity seen in hilar renal lymph on constriction of the renal artery may be a concentration effect; a constant amount of renin may be added to a decreased volume of lymph. This would give rise to a greater constrictor activity being assayed in the samples collected while the artery was constricted as compared to those lymph samples collected during the control period. However, an increased rate of movement of a pressor substance into the lymph would be indicated if similar vasopressor activity were found in both the constriction and first post-constriction lymph samples. The rate of lymph flow of the first post-constriction sample of lymph is considerably greater than the rate of lymph flow while the renal artery is constricted. Equal pressor activity in these samples of lymph would indicate an increased rate of secretion of pressor substance into the post-control lymph. The first post-control lymph sample, (sample PC_1, Figure 7) treated with calcium-complexed EDTA has an increased pressor activity equal to the activity seen in the corresponding lymph collected while the artery was constricted. However, this is not true for the first post-control lymph sample that was treated with uncomplexed EDTA. It would appear, therefore, that the decreased lymph flow rate that occurs with constriction of the renal artery is an important factor in determining the increased vasopressor activity that appears in the lymph collected at that time.

If renin is, in fact, present in the renal hilar lymph the

question arises as to just how and where renin gains access to the lymph. Renin is thought to be secreted by the cells of the juxtaglomerular apparatus (JGA) (4, 12). The JGA is the name given by Goormaghtigh to the specialized cells of the media of the afferent arteriole of the glomerulus. The cells of the JGA are afibrillar, epithelial cells which contain granules which apparently contain renin (7, 21). However, many investigators who have studied the distribution of renal lymphatics claim that there are no lymphatic capillaries associated with glomeruli or the afferent or efferent arteriole of the glomerulus (8, 34). If this is so, the renin present in renal lymph must have been previously diluted by the blood plasma, tubular and interstitial fluid from which the lymph is derived (14). Therefore, there may exist within the interstitial fluid of the kidney, local concentrations of renin of a greater concentration than that seen in renal lymph. Lever and Peart (17) have suggested that local concentrations of renin within the kidney may be of importance in the interrelationships that exist between renin, angiotensin and aldosterone.

The Pump-perfusion Experiments

These experiments were carried out to test the effect of local changes in renal blood flow and/or oxygen tension upon the release of renin into the renal venous blood. The results of these experiments show that samples of control plasma contain vasopressor activity which may or may not be slightly increased due to the surgical manipulation of the kidney. Subjecting the experimental kidney to 17 minutes of severe hypoxia (renal artery oxygen tension: 0-13 mm Hg) neither increased nor decreased the vasopressor activity of the renal venous blood. These

results are comparable to those of Skinner, McCubbin and Page (41) who noticed a continuous secretion of a slight amount of renin during control periods of similar experiments. These authors also reported a lack of increased renin secretion in animals in which the systemic arterial oxygen saturation was reduced to 50-70%.

The results of the present study also indicate that reduction of the blood flow to the experimental kidney is followed by increased vasopressor activity appearing in the renal venous effluent. The increased vasopressor activity appears in the renal venous plasma even when the kidney is hypoxic. This agrees with the fact that increased vasopressor activity was observed in the blood sample obtained after complete occlusion of the renal artery during the natural flow experiments. However, the finding of increased vasopressor activity concomitant with hypoxia appears somewhat contradictory to the findings of Skinner, McCubbin and Page (41) that complete occlusion of the renal artery is not accompanied by a release of renin into the renal venous blood. These authors postulated that the secretion of renin is a process which requires oxygen. The results of the present study do not support this hypothesis.

In one series of experiments (Figure 8) reduced flow to the experimental kidney was maintained for a period of 31 minutes. During the last 15 minutes of the period, the kidney was also made hypoxic. Yet the vasopressor activity of the plasma collected during the first 16 minutes was the same as the activity found in the plasma drawn after the condition of hypoxia was combined with low flow. If oxygen markedly facilitated the secretion of renin, the vasopressor activity of the blood drawn during the period of combined low flow and hypoxia would be

decreased. However, the constrictor activity of the blood obtained after 31 minutes of low flow might have been greater had not hypoxia been imposed upon the kidney.

In an effort to determine what effect the experimental sequence had on the secretion of the pressor agent, experiments were done (Figure 9) in which a low blood flow to the experimental kidney was maintained for 29 minutes. During the first 14 minutes the kidney was also subjected to hypoxia. If hypoxia inhibits renin secretion, the sample obtained during the period of low blood flow would have a greater pressor activity than the blood sample drawn during the period of hypoxia plus low blood flow. While the average pressor response obtained on assay of the plasma drawn during the period of low blood flow was greater than the response to plasma obtained during the period of hypoxia plus low blood flow, there was considerable overlap in the response of the assay animals to the injection of these two plasma samples (sample LF + 5% CO_2-95% N_2, and sample LF, Figure 9). This overlap, coupled with the fact that there was apparently no change in the secretion of pressor material during hypoxia in the presence of high renal blood flow (sample 5% CO_2-95% N_2), leads to the postulation that the secretion of the pressor agent is unaffected by severe hypoxia.

In this same series of experiments a sample of blood (sample P.C. 30 Sec, Figure 9) was obtained 30 seconds after the flow was returned to control level from the level of reduced blood flow. The vasopressor activity of this sample is only slightly greater than the control sample taken before blood flow was reduced. This indicates that the increased pressor actively seen in the samples taken during reduction in the renal

blood flow was due to the concentrating effect of the reduced blood flow.

Control of Renin Secretion

This study shows that the appearance of increased pressor activity in the renal hilar lymph or renal venous blood follows a reduction in the perfusion pressure or blood flow to the kidney. Several groups of investigators have studied the mechanisms underlying the secretion of renin by the kidney (41, 45, 46, 47). Page and his co-workers, (41) state that the secretion of renin is controlled by a baroreceptor mechanism within the renal pyrenchyma. The normal level of renal perfusion pressure is such that renin secretion from the kidney is minimal. A decrease in perfusion pressure is sensed by the renal pressostat, and renin secretion is increased. The increased renin secretion acts to increase the concentration of circulating angiotensin. The increased angiotensin raises the renal perfusion pressure. The increased perfusion pressure, in turn, is sensed by the renal pressostat which decreases renin output. Tobian (44, 46) also believes that the kidney is capable of sensing changes in the renal perfusion pressure and altering the secretion of renin accordingly. He suggests that the juxtaglomerular cells act as stretch or volume receptors which function in the control of renin secretion. Renal artery constriction decreases the stretch of the afferent arteriole and the cells of the JGA which are contained within the arteriole. When this occurs renin secretion is increased. Conversely, increased renal perfusion pressure increases the stretch of the juxtaglomerular cells and renin secretion declines.

The results of the present study do not support the view that renin secretion is regulated solely by changes in the mean perfusion

pressure of the kidney. When the experimental kidney was subjected to severe hypoxia, the renal perfusion pressure fell, while the total renal blood flow was held constant. Yet samples of renal venous blood obtained during this period of renal dilation did not contain a greater pressor activity than the control samples. It might be that oxygen is required for renin to be secreted. However, when renal perfusion pressure and renal blood flow were reduced simultaneously in these same experimental animals, increased vasopressor activity appeared in the renal venous blood, whether or not the kidney was hypoxic. This finding would suggest that a decrease in renal blood flow may in some way influence the secretion of renin by the experimental kidney. Vander and Miller (47) decreased the renal perfusion pressure of dogs to 90 mm Hg and observed an increase in renin secretion. Administration of diuretics to the experimental animals reversed the effect of decreased perfusion pressure; the plasma pressor activity declined toward control levels. These authors suggested that renin secretion is regulated by the renal tubular handling of sodium and water. A decrease in perfusion pressure is accompanied by a decrease in the glomerular filtration rate (GFR). The decreased GFR allows more time for sodium and water reabsorption from the renal tubule. The increased reabsorption of sodium stimulates renin secretion. Administration of diuretics during the period of reduced renal perfusion pressure, increases the rate of tubular flow and, therefore, reduces sodium reabsorption and renin secretion.

Sodium reabsorption appears to be linked to the secretion of renin at the level of the macula densa (43, 47). The macula densa is that portion of the distal tubule which abuts the afferent (and sometimes efferent) arteriole of the parent nephron (21). Unlike the remainder of

the cells of the distal tubule, the cells of the macula densa are closely packed. Between the juxtaglomerular cells and the macula densa the basement membrane is very thin with the Golgi apparatus lying against the basement membrane (12, 22). This arrangement of the macula densa and the juxtaglomerular cells would facilitate the movement of sodium from the tubular fluid at the level of the macula densa into the juxtaglomerular cells (43).

Stamey (27) postulates that renin secretion is a function of the sodium concentration surrounding the macula densa. Decreased renal perfusion pressure would increase the sodium reabsorbed by the macula densa and, this, in turn, would increase renin secretion as described in the experiments of Vander sited above. Increased renin secretion would constrict the efferent arteriole and cause an increase in the GFR which would reduce sodium reabsorption with a resultant inhibition in renin secretion.

Renal hypertensive disease sometimes occurs in cases where the renal perfusion pressure to the involved kidney is so reduced that filtration has ceased (43). It is difficult to reconcile an increased reabsorption of sodium in the absence of any filtered sodium. Indeed, in the present study the renal perfusion pressure in ten experiments was reduced to a level of 27-29 mm Hg. Glomerular filtrate would not be formed at this level of pressure. Yet renin secretion apparently continues and may be increased in these kidneys. While these results cannot be explained by an increased concentration of sodium at the macula densa, they could be explained on the basis of the filtered load of sodium. Gross, Brunner and Ziegler (9) speculate that the secretion of

renin is controlled by the sodium load presented to the macula densa. Either a decrease in GFR or a decrease in plasma sodium would produce a decrease in the filtered load of sodium presented to the macula densa. This decrease would stimulate renin secretion. According to this theory, renin secretion would increase in the absence of filtration in the kidney.

CHAPTER V

SUMMARY AND CONCLUSIONS

Renal venous plasma and/or renal hilar lymph was collected from 19 dogs before, during and following partial occlusion of the renal artery. Plasma and lymph samples were assayed for pressor activity by i.v. injection of the sample into an appropriately prepared rat. Both the renal venous plasma and renal hilar lymph were vasopressor in the rat. Increased vasopressor activity appeared in the plasma and lymph following partial occlusion of the renal artery. Release of the arterial occlusion saw a gradual decline in the vasoconstrictor activity of the plasma and lymph to control levels. Per unit volume, the pressor activity of the renal hilar lymph was always greater than the corresponding plasma sample.

Samples of renal venous plasma were obtained from ten additional dogs in which the left kidney was pump perfused through an extracorporeal lung circuit. The lung was ventilated with various gas mixtures. In this way it was possible to locally alter blood flow and/or the oxygen tension of the blood entering the experimental kidney. Assay of plasma samples obtained during periods of altered renal blood flow and/or altered renal artery oxygen tension indicate: (1) with flow held constant at control levels, the near absence of oxygen in the perfusing blood does not increase the output of pressor substance, (2) reduction in renal blood

flow (and, therefore, also renal blood pressure) is followed by increased pressor activity appearing in renal venous blood even during an extreme reduction in the oxygen tension of the perfusing blood. Therefore, under the conditions of our experiments, oxygen per se is not an important determinant of renal venous pressor activity.

The vasopressor substance of the present study is most likely angiotensin II produced by the renin-angiotensin system. The results of this study support the thesis that the secretion of renin is not solely controlled by a renal pressostat. Changes in renal blood flow, independent of a change in renal blood pressure, may alter renin output.

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