EFFECT OF RENIN ON PROTEINURIA AND PAH CLEARANCE AT LOW PLASMA LEVELS

J. LEONARD BRANDT AND JOHN G. GRUHN

From the Department of Medicine, Long Island College of Medicine
BROOKLYN, NEW YORK

In 1940 Pickering and Prinzmetal (1) reported that strongly pressor doses of renin profoundly influenced and modified renal function in the rabbit. This modification was manifested in a marked diuresis, with increased excretion of sodium and chlorides. These changes in renal function were attributed to a direct action of renin on the tubules; the changes persisted as long as renin was given and after tachyphylaxis had abolished the pressor action. Since renin may have a primary function of altering kidney activity and metabolism, we have extended these studies to include other phases of renal function in the rabbit. Particular attention was paid to glomerular damage, by studying proteinuria and determining changes in permeability of the glomerular membrane to injected hemoglobin. Preliminary experiments confirmed the observation that the diuresis resulting from the injection of pressor doses of renin in the rabbit was accompanied by an increased proteinuria (1).

METHODS

All observations included a control period and the period of maximum renin diuresis.

Unanesthetized male rabbits weighing between 1.5 and 3.0 kgm. were used throughout. The animals were kept on their usual diet of commercial rabbit chow up to the evening preceding an experiment when all food was withdrawn and water allowed ad libitum. The morning of the experiment, the animal was hydrated with 50 to 100 cc. of warm tap water by stomach tube. Approximately one hour before the start of a control urine collection period, 500 mgm. of creatinine dissolved in 10 cc. of normal saline was administered subcutaneously. This produced initial blood levels between 10 to 20 mgm. per cent. The animal was then tied to a board and his bladder thoroughly emptied and washed, using a No. 8 F catheter, to insure that subsequent protein determinations did not reflect mucin and detritus from the lower urinary tract. The catheter was cut short to remove as much dead space as possible in the urine collections and was allowed to remain in place throughout the period of observation.

To determine the clearance of para-aminohippuric acid, 250 mgm. PAH in 10 cc. saline was given subcutaneously approximately 15 minutes before the expected start

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2 Animals that were to receive hemoglobin were given 0.5 gram of sodium bicarbonate in the water.

3 Supplied by Sharpe and Dohme, Inc. as sodium para-aminohippurate.
of a control urine collection period. This gave initial blood levels between 3.5 to 8.0 mgm. To study hemoglobin permeability, the procedure of Monke and Yuile (2) was used. Initial blood levels of hemoglobin between 250-500 mgm. per cent were obtained by the intravenous injection of 350 to 750 mgm. of rabbit hemoglobin, as 10 to 20 per cent solution, 10 minutes before the start of the control period. Hemoglobinuria was apparent about 7 minutes following the injection.

Urine was collected directly into graduates and midperiod blood specimens obtained by direct cardiac puncture. Particular care was taken to prevent hemolysis.

Following a control period in which specimens for creatinine clearance, PAH clearance, hemoglobin permeability and urinary proteins were collected, a few minutes were allowed to elapse during which time the bladder was again washed and emptied. The animal was then given 10 mgm. of renin in 2 cc. of physiological saline intravenously. Gross inspection of the rate of urine flow was found to be a satisfactory method of estimating the period of peak diuresis, during which the post-renin observations were made. These peak periods usually occurred about 20 minutes after the renin injection. No animal was used more than once.

Determinations of creatinine in plasma and urine were done by the method of Folin and Wu (3); PAH determinations in plasma and urine by the method of Smith, et al. (4). The determination of hemoglobin concentrations in both the hemoglobin solution used for the injection, and standard solutions used for determining the plasma and urine hemoglobin concentrations were done by the iron method of Wong (5). The plasma and urine hemoglobin concentrations were determined by a modification of the cyanmethemoglobin method of Evelyn and Malloy (6). A Klett-Summerson photoelectric colorimeter was used for all color reactions.

In determining proteinuria, the Shevky-Stafford method (7) using Tsuchiya’s reagent was used. When hemoglobin was also present in the urine, the volume precipitate for hemoglobin was subtracted from the total using a graph of precipitates from known concentrations of hemoglobin. It was found that weight for weight, both hemoglobin and albumin gave about the same amount of precipitate with Tsuchiya’s reagent. In animals made hemoglobinuric, the high control period urine concentration of hemoglobin might account for perhaps nine tenths of the total protein (hemoglobin and albumin) precipitated. The albumin determination therefore was below the limits of desired accuracy, and control period proteinuria is recorded as less than 0.3 mgm. protein/minute. The calculated values were 0.1 to 0.3 mgm./minute. During the period of renin diuresis, the urinary hemoglobin accounts for about one half or less of the total precipitate, and determinations of urinary protein after subtraction of the hemoglobin precipitate is accurate. The validity of these conclusions was borne out by data obtained in animals that were not given hemoglobin, and their urinary protein per minute during a control period and during renin diuresis determined gravimetrically. The gravimetric figures were in concordance with the Shevky-Stafford method of quantitating urinary protein in the presence of hemoglobin, during renin diuresis.

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*Hog renin supplied through the courtesy of Dr. K. Kohlstaedt of Eli Lilly Co. A 10 mgm. dose tested in anesthetized animals by carotid artery B.P. gave rises in pressures of 30-40 mm. Hg which reached a peak within 30 seconds and returned to base-line in about 20 minutes.*
In all animals a simple qualitative Benedict's test for sugar was done on the control urines and the urine obtained during peak renin diuresis.

Six animals were killed following an experiment and their kidneys removed for microscopic examination.

RESULTS

In carrying out these experiments, a total of 12 animals were used. As had been mentioned, not all procedures were carried out in all animals simultaneously. Most observations were made during a control period and during peak renin diuresis. Of the 12 animals, 8 gave a minimum of about four-fold increase in urine flow per minute during renin diuresis over control periods, although there was a definite increase in flow in all animals. Nine sets of observations of the glomerular filtration rates were made. Eight sets of observations of the clearance of PAH were made, using subcutaneous injections of NapAH. Two additional animals were observed for PAH clearance using Forster's (9) intravenous infusion technique. These animals gave very similar results to the subcutaneous injection method, and so intravenous infusion was abandoned since it was felt that this would alter urine flows. A total of five observations of proteinuria were done, and the accuracy checked against observa-
tions in two animals not given hemoglobin. In these, urinary proteins were determined gravimetrically.

In table I are recorded the typical observations made on the 12 animals. The upper figure of each pair is the control datum, and the lower is the datum during renin diuresis.

Column B records the creatinine clearances. These clearance figures are a measure of the glomerular filtration rate in the rabbit, as has been pointed out by Kaplan and Smith (8), and correspond with the thiosulphate clearance (10), as we were able to observe in 10 experiments on rabbits.

In column C are recorded the clearances of PAH during a control period and during renin diuresis in eight animals. The subcutaneous method gave results similar to those with continuous infusion. Inasmuch as extraction ratios were not done, it is not certain that the clearance figures truly reflect changes in effective renal blood flows. However, the work of others (12) indicates that the effective renal blood flows are diminished with renin, as would be expected with a vasoconstrictor.

Column A records the changes in urine flows during the control periods and during renin diuresis.

Column D lists the quantitative protein determinations. Animals 1 and 3 received no hemoglobin; animals 4, 8, and 11 received hemoglobin. These quantitative protein determinations were done by the Shevky-Stafford method. Animals X and Y received no hemoglobin and their urinary proteins were determined gravimetrically. In animal X, during the decline in diuresis, about 80 minutes after the injection of

Fig. 1. FUNCTIONAL CHANGES in the kidney during renin diuresis expressed as percentage change from control period; columns 6 and 7 based on data of Pickering and Prinzmetal (1). 1, glomerular filtration rate; 2, PAH clearance; 3, urine flow/min.; 4, proteinuria/min.; 5, glomerular permeability to hemoglobin; 6, urinary sodium/min.; 7, urinary chloride/min.
renin, when the urine flow had dropped from 3.7 cc./minute at its peak to 0.8 cc./minute, proteinuria also dropped from 1.1 mgm./minute to 0.07 mgm./minute.

Column E records the results of the Benedict's test for sugar in the control period urine, and in the urine during peak renin diuresis.

Column F records the hemoglobin permeability.

**DISCUSSION**

Addis (13) pointed out that proteinuria is normal in the human and is pathological only when present in excess amounts. Oliver (14) notes the presence of protein molecules in the tubules and tubular cells of what are considered normal mammalian kidneys, using protein-dye combinations.

Methods of studying proteinuria dynamically had never met with great success, until Dock (15) suggested that the hemoglobin molecule might serve the purpose of ruling out changes in the permeability of the glomerular membrane to account for changes in protein excretion, when hemoglobinuria and albuminuria are studied simultaneously. The hemoglobin molecule has 97 per cent the molecular weight of the albumin molecule, and above Tm values, is cleared by the kidney in a definite and predictable proportion of the filtration rate. This proportion expressed in per cent is the ratio of the simultaneous clearance of hemoglobin above Tm values to the glomerular filtration rate and has been designated by Monke and Yuile (2) as the hemoglobin permeability. This figure is interpreted by them to indicate the per cent of pores of the glomerular membrane large enough to allow the passage of an undisassociated hemoglobin molecule. This investigation showed (column F, table 1) that under normal conditions the glomerular membrane of the rabbit has about 4 to 5 pores per hundred that are large enough to permit the passage of hemoglobin molecules. With these basic facts one can then infer whether proteinuria is the result of a 'damaged' (more permeable) glomerulus or possibly is the result of some other factors acting on or in the kidney.

It is to be understood that although the hemoglobin and the albumin molecules have about the same molecular weight it does not follow that their glomerular permeabilities are alike, since shape and electrical charge may be different. Hemoglobin permeability is about 5 per cent and albumin permeability less than 1 per cent (15, 16).

From the average glomerular filtration rates for the animals examined (8.3 cc. before renin and 8.6 cc. after renin), it is apparent that the filtration rate is unchanged but there is a marked increase in urine flow (average 0.31 cc. before renin and 1.78 cc. after renin). Diuresis is the result of diminished tubular reabsorption of water. The only other explanation for the diuresis lies in the possibility that it is osmotic in nature. That such is not the fact is adequately established by Pickering and Prinzmetal (1). The clearances of PAH (column C, table 1) indicate that there is probably a significant fall in the effective renal plasma flow, which, in conjunction with an insignificant change in the glomerular filtration rate, indicates a rise in the filtration fraction and efferent arteriolar constriction. Could the resultant increase in intraglomerular pressure account for the proteinuria? The data in columns D and F indicate that in spite of this increased pressure the glomerular membrane is no more
permeable to the hemoglobin molecule during renin diuresis. The clearance of hemoglobin varies with the filtration rate and there is no change in ratio of hemoglobin clearance to the creatinine clearance. It seems safe to conclude from this that no greater amounts of protein are appearing in the glomerular filtrate during renin diuresis than during the control period. The proteinuria must therefore be the result of diminished tubular reabsorption of protein. This depression of tubular reabsorption is a purely physiological and transient phenomenon, since, the subsidence of the diuresis is accompanied by a decrease in the amount of protein appearing in the urine. One may indulge in the luxury of calculating the amount of protein in rabbit glomerular filtrate, assuming that the concentration in glomerular filtrate was constant and no albumin was reabsorbed during renin diuresis. Such calculations, based on table 1, give values of 11 to 33 mgm. per cent, with 4 out of 5 animals showing less than 20 mgm. per cent. These figures are concordant with experiments in which the tubules have been paralyzed by cold and a glomerular filtrate obtained (15).

In spite of the general depression of tubular reabsorptive capacity during renin diuresis, glucose does not appear in the urine. It is noted in column E that all but one animal had a slight glycosuria during the control period. This glycosuria has been interpreted as probably a psychogenic effect rather than anything pathological. The absence of detectable glycosuria or increase in hemoglobinuria during renin diuresis, when tubular reabsorption of sodium, chlorides, water (1) and protein are depressed, suggest a possible dissociation of tubular activity.

Renin can be isolated from the kidneys of normal animals and produces its pressor effects by catheptic action on plasma protein. It may be that its action on the renal tubule is of far greater biologic importance than its participation in the pressor response to renal ischemia just as the effect of pitressin on the tubule seems more important than its pressor action. By reducing the work of reabsorption at times when renal blood flow is greatly reduced, renin may play a part in the reduction of total renal metabolism observed by Van Slyke and his associates (17). In the rabbit, no rise in arterial pressure was noted after removal of a clamp causing complete renal artery occlusion for two hours; and 23 of 30 animals survived (18).

Microscopic examination of the kidneys of 6 rabbits used in these experiments failed to show evidence of any renal damage.

SUMMARY

1. Strongly pressor doses of renin in the unanesthetized rabbit produce a marked diuresis unaccompanied by alterations in the glomerular filtration rate. As shown by others (1) the diuresis is due to an inhibition of tubular reabsorption of water and is associated with inhibition of reabsorption of sodium and chloride.

2. Strongly pressor doses of renin given to the hydrated unanesthetized rabbit produce a slight decrease in PAEI clearance and a marked increase in proteinuria. The increased proteinuria is not accompanied by any significant change in the permeability of the glomerular membrane to injected hemoglobin, and is apparently due to diminished tubular reabsorption of protein.

3. The marked alterations in renal function produced with pressor doses of renin are transient and are not accompanied by any lesions that can be seen microscopically.
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REFERENCES