Plasma Renin Substrate Changes in Experimental Uremia[1] (35184)

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It is well recognized that plasma renin substrate concentration increases markedly after nephrectomy (1, 2). In addition, it has been reported that plasma renin substrate concentration is increased during renal damage or loss of renal function due to mercuric chloride poisoning or bilateral ureteral ligation (3). It has also been reported that renin substrate increases terminally in malignant hypertension in humans (4, 5). These situations are associated with the development of uremia secondary to renal damage. To investigate the possibility that the changes associated with the development of uremia rather than tubular damage lead to increased renin substrate concentration, measurements of renin substrate have been made in dogs with severe renal tubular damage secondary to ureteral ligation and renal ischemia and in dogs with the ureters shunted into the inferior vena cava. The experimental plan was to produce the same level of uremia with and without severe tubular damage.

Materials and Methods. Surgical preparation. Eight mongrel dogs weighing 14–22 kg were subjected to ureteral ligation and temporary renal ischemia. Under sterile conditions and pentobarbital anesthesia (30 mg/kg) the ureters were ligated and sectioned and a serrefine clamp was placed on each renal artery and allowed to remain for 2 hr. After the 2-hr period of renal ischemia, the clamps were removed from the renal arteries and the dogs were allowed to recover from surgery.

Seven dogs weighing 14–22 kg were subjected to uretero–vena cava shunting. Under pentobarbital anesthesia, the ureters were shunted into the inferior vena cava via a lumbar vein. The shunt consisted of a short length (approx 5 cm) of polyvinyl choride catheter (no. 8 French infant feeding tube, American Hospital Supply) which was tied into the proximal end of the cut ureter and lumbar vein with 2-0 silk. The distal end of the ureter was ligated.

Experimental protocol. In both groups of dogs a venous blood sample was obtained before surgery for measurement of plasma renin substrate concentration, plasma renin activity, plasma sodium and potassium concentration, and blood urea nitrogen (BUN). The dogs were brought to the laboratory each day for 2 days after surgery and venous blood samples were taken for measurement of the above memtioned parameters. After sampling was completed, the animals were given 200 ml of 20% dextrose in water each day. On the third day after surgery, the dogs with uretero–caval shunts were anesthetized with sodium pentobarbital and the shunts were opened and visually observed for patency. These dogs were then killed with a large dose of anesthetic. The dogs with ureteral ligation and renal ischemia were maintained for use in another experiment.

Analytical procedures. Ten-ml blood samples were collected in cold tubes with 0.1 ml of 10% ethylenediaminetetraacetate (EDTA). The plasma was separated by centrifugation and stored frozen until it was assayed for renin substrate concentration and renin activity. For measurement of substrate concentration, a 1:10 dilution of dog plasma was made with phosphate buffer at pH 5.3. To 2 ml of this solution, 0.1 ml of a 1:10 dog renin solution (approximately 1.0 Goldblatt unit/ml) was added in addition to 0.05 ml of

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1:100 diisopropyfluorophosphate (DFP), 0.05 ml of saturated sodium chloride, and 0.1 ml of a 10% solution of EDTA. This mixture was incubated at 37° for 3 hr. The tubes were placed in boiling water for 10 min and then cooled in ice. The volume was adjusted with phosphate buffer (pH 8.3) to 4 ml and the solution was mixed and centrifuged. The supernatant was used for assay. Several samples treated in this manner were incubated for varying lengths of time (1–6 hr) to insure that all substrate had been converted by 3 hr and that angiotensinase activity had been inhibited. In all cases, the generated angiotensin reached maximal values by 1–2 hr and did not decrease with longer incubation periods. The method of Schneider *et al.* (6) was used for determination of plasma renin activity.

The angiotensin generated by these procedures was assayed in the vagotomized, pentolinium treated rat; val^5-angiotensin II amide (Hypertensin, Ciba) was used as the standard. Renin activity and substrate concentration are expressed as nanograms of angiotensin per milliliter of plasma.

Plasma electrolytes were measured by flame photometry and BUN by a standard Technicon AutoAnalyzer technique.

Results. In the eight dogs that were subjected to ureteral ligation and renal ischemia, plasma renin substrate concentration was 576 ± 119 (SEM) ng of angiotensin/ml of plasma during the control period. On day 1 after ureteral ligation and renal ischemia, plasma renin substrate concentration was 1021 ± 237 ($p<0.02$) and on day 2 plasma renin substrate concentration was 878 ± 182 ($p<0.01$) (Table I). These increases in renin substrate concentration were associated with increases in plasma renin activity from 4.7 ng of angiotensin/ml of plasma during control observations to 24.2 ± 6.1 on day 1 ($p<0.05$) and 17.2 ± 3.7 on day 2 ($p<0.01$) after the procedure of ureteral ligation and renal ischemia.

On the basis of paired statistical analysis, uretero–caval shunting in seven dogs resulted in small but significant increases in plasma renin substrate concentration. Before shunting in this group of dogs, the plasma

TABLE I. Summary of All Data Collected Before and on Days 1 and 2 After Production of Uremia.

	Ureteral ligation + temporary renal ischemia			Uretero–caval shunt		
	Control	Day 1	Day 2	Control	Day 1	Day 2
Plasma renin substrate conc (ng of angiotensin/ml)	576 ±119[a]	1021 ±237	878 ±182	504 ± 53	573 ± 48	603 ± 59
Plasma renin activity (ng angiotensin/ml)	4.7 ± 0.7	24.2 ± 6.1	17.2 ± 3.7	5.5 ± 1.2	12.4 ± 3.5	7.9 ± 1.1
BUN (mg/100 ml)	17 ± 1.4	79 ± 6.2	123 ± 8.2	18 ± 1.8	93 ± 16.2	142 ± 17.1
Plasma electrolytes (mEq/liter) Na	142 ± 0.8	142 ± 1.7	139 ± 0.9	146 ± 1.2	139 ± 1.4	138 ± 2.1
K	4.4 ± 0.2	5.0 ± 0.2	5.4 ± 0.3	4.4 ± 0.2	5.0 ± 0.2	5.9 ± 0.4

[a] Mean ± standard error of mean.

renin substrate concentration was 504 ± 53 ng of angiotensin/ml of plasma. On day 1 after surgery, plasma renin substrate concentration was 573 ± 48 ($p<0.05$) and on day 2 plasma renin substrate concentration was 6.03 ± 59 ($p<0.02$). Plasma renin activity was 5.5 ± 1.2 mg of angiotensin/ml of plasma before surgery and 12.4 ± 3.5 on day 1 after surgery ($p>0.1$) and 7.9 ± 1.1 on day 2 ($p<0.4$). All of these dogs exhibited urine flow when the shunts were opened after all blood samples had been collected.

Table I summarizes the measured variables in the two groups of dogs. On days 1 and 2 after the surgical procedure, plasma sodium and potassium concentrations and BUN were not significantly different between the two groups.

When the increases in plasma renin substrate concentration above their respective control values were compared between the two groups of dogs, a highly significant difference was observed (Fig. 1). The dogs that had ureteral ligation plus temporary renal ischemia had much larger increases in renin substrate concentation than did the dogs with uretero–caval shunting. On day 1 after surgery, the difference between the two groups was 376 ng of angiotensin/ml of plasma ($p<0.025$) and on day 2 the difference was 220 ng of angiotensin/ml of plasma ($p<0.01$).

Discussion. This study demonstrates that a large increase in plasma renin substrate concentration occurred in dogs after damage to the kidney that resulted in severe tubular necrosis (7–10). It is well known that plasma renin substrate concentration increases after nephrectomy (1, 2). A possible explanation of the increase in renin substrate concentration in this situation is loss of the source of renin and hence substrate is not converted to angiotensin I and accumulates in the systemic circulation. In this study, plasma renin substrate increased in association with large increases in plasma renin activity. This finding confirms the results of Carretero and Gross (11) in rats. Since plasma renin substrate concentration can be elevated in the presence of high or low to absent renin (3, 10), it is unlikely that the level of renin substrate in the plasma is much affected by the level of circulating renin.

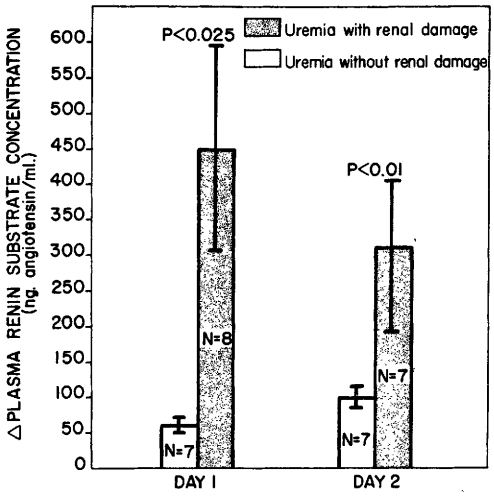

FIG. 1. Comparison of the increase in plasma renin substrate concentration above control values on days 1 and 2 after surgery for dogs with uretero-caval shunts (open columns) and dogs subjected to ureteral ligation and renal ischemia (stippled columns); mean increase ± standard error of the mean.

It had been previously recognized that severe renal tubular damage secondary to administration of nephrotoxic chemicals in the rat resulted in increased renin substrate concentration (3). Terminal malignant hypertension is also associated with elevated substrate levels in humans (4, 5). Both of these conditions, as well as nephrectomy, result in uremic changes associated with loss of renal function. This study demonstrates that uremia itself was not responsible for the very large increases in renin substrate concentration associated with loss of renal function. Although small but significant increases in renin substrate concentration were observed after uretero–caval shunting, these were of minor significance when compared to the increases observed after renal tubular damage. It has been shown by Haynes *et al.* (12) that ACTH increases plasma renin substrate concentration. It is possible that the small increases in substrate levels observed after uretero–caval shunting were due to stress induced ACTH release secondary to the surgical procedure. In addition, some renal tubular damage may have resulted from the shunting procedure due to venous pressure or overtightening of the ligature securing the

shunts which could have resulted in possible hydronephrotic changes.

Since measurement of plasma renin substrate concentration depends upon an indirect assay method utilizing the generation of angiotensin, it is possible that an inhibitor of the renin–angiotensinogen reaction could be lost after nephrectomy or severe renal tubular damage (3, 13). This explanation appears unlikely, however, since Ostrovsky *et al.* (14) have reported that plasma levels of the phospholipid renin preinhibitor were not reduced 48 hr after nephrectomy.

Blaine and Davis (10) have shown that in dogs with ureteral ligation and temporary renal ischemia, the kidneys were no longer capable of glomerular filtration. Hence, in the present study, a second possible contrast between the two groups of dogs can be made. In the dogs with uretero–caval shunts, filtration continued (as evidenced by the urine flow observed upon opening the shunts) while in the dogs with ureteral ligation and temporary renal ischemia, filtration ceased. Thus, the possibility exists that the concentration of renin substrate in the plasma might, in some manner, be associated with glomerular filtration. This mechanism would require that the renal tubular epithelium be sensitive to changes in filtration rate or possibly the concentration of some substance in the glomerular filtrate and that these changes in some manner alter renin substrate production by the liver. This explanation would satisfy the findings in nephrectomy, severe renal tubular damage, ureteral ligation, and in malignant hypertension since all of these conditions are associated with decreased or absent glomerular filtration.

Summary and Conclusions. The effect of uremia on plasma renin substrate concentration was investigated in dogs with and without severe renal tubular damage. Dogs with renal tubular damage secondary to ureteral ligation and renal ischemia had markedly elevated plasma renin substrate concentration. These elevations in plasma renin substrate concentration were associated with elevated plasma renin activity. Another group of dogs with uretero–vena cava shunts exhibited the same degree of uremia but had a much smaller increase in plasma renin substrate concentration and no change in plasma renin activity. It is concluded that uremia *per se* did not stimulate the very large increase in plasma renin substrate concentration observed after renal tubular damage. The increased renin substrate concentration was associated with loss of renal tubular function and cessation of glomerular filtration. These results support the concept that plasma renin substrate concentration may, in part, be controlled by changes in glomerular filtration rate or alterations in renal tubular function.

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