Cellophane Perinephritis Hypertension and Its Reversal in Rabbits

EFFECT ON PLASMA RENIN, RENIN SUBSTRATE, AND RENAL MASS

By Duncan J. Campbell, Sandford L. Skinner, and Allan J. Day

ABSTRACT

Plasma renin activity and renin substrate levels were measured during the development of cellophane perinephritis hypertension induced by either a bilateral renal wrap procedure or a wrap-nephrectomy procedure in 28 rabbits. Unoperated and unilaterally nephrectomized controls were also studied. Plasma renin concentration was derived from plasma renin activity and plasma renin substrate levels using a measured $K_m$ of $2.3 \times 10^{-9} M$. All surgical operations were followed by a transient increase in plasma renin substrate levels and a fall in plasma renin concentration. Unilateral nephrectomy in normotensive controls suppressed plasma renin activity and concentration for a 20-day period. Similar suppression occurred during the onset of hypertension in rabbits subjected to the wrap-nephrectomy procedure. Decapsulation accompanied by a fall in blood pressure again suppressed plasma renin activity and concentration. With bilateral wrapping, hypertension developed gradually without a change in renin levels. Decreased renin secretion during the evolution of perinephritis hypertension probably reflects a decreased renal excretory capacity for salt and water that overrides the renin-releasing effect of renal compression.

KEY WORDS: renin secretion, renin kinetics, unilateral nephrectomy, sodium excretion, blood pressure measurement

The production of chronic hypertension in experimental animals by the technique of cellophane perinephritis was first described by Page in 1939 (1), but compared with hypertension induced by renal artery constriction (2) or by salt loading accompanied by reduced renal mass (3), its pathogenesis has been less extensively studied (4). With perinephritis, as with other forms of experimental renal hypertension (5, 6), it seems likely that the function relating salt and water output to renal perfusion pressure is displaced so that less output is possible at normal pressures. The consequent expansion of extracellular fluid volume ultimately leads to an increase in peripheral resistance and arterial blood pressure. Experimental proof of such a process in hypertensions of slow onset, such as perinephritis, is difficult to obtain: the degree of volume expansion required to initiate and maintain the autoregulatory increase in peripheral resistance may be beneath the sensitivity of the methods available for its detection (6, 7). In hypertension of more rapid onset following renal artery constriction, expansion of extracellular fluid volume has been conclusively shown when all renal tissue is subject to reduced perfusion, but this detectable expansion is transient (8-10).

Studies of the renin-angiotensin system in hypertension have been primarily addressed to the question of whether the renal manipulation increases renin secretion and leads to initiation and maintenance of hypertension through vasoconstrictor and sodium-retaining effects (4, 11, 12). Little attention has been paid to the likelihood that any sodium retention occurring as a result of the renal manipulation would lead to suppression of renin secretion provided that there has been little interference with the renin secretory mechanism (13). Indeed, the hypertension itself by increasing renal perfusion pressure would probably have the same effect (14). In support of such a possibility, Mogil et al. (15) have demonstrated a fall in plasma renin activity in perinephritis hypertension in dogs; however, Lewis and Lee (16) could not detect a decrease in rabbits. The present work confirmed that suppression of renin secretion could occur during perinephritis hypertension in rabbits and sought to identify the relative importance of enzyme and substrate concentrations, renal mass,
and renal perfusion pressure in the production of this effect.

Methods

Twenty-eight New Zealand white rabbits were used in the hypertension experiments, eight New Zealand white rabbits were used as unoperated controls, and six crossbreeds were used to study the effects of unilateral nephrectomy alone. All rabbits were 10-16-week-old males that weighed 2-2.5 kg at the commencement of the experiment. They were maintained on Barastoc pellets containing 0.3% sodium chloride and tap water. The experimental design permitted measurement of blood pressure, plasma renin activity, plasma renin concentration, plasma renin substrate, and plasma urea before, during, and after the production of hypertension. In all rabbits subjected to surgery (Figs. 4-7), the mean preoperation values were obtained from several control measurements collected from each rabbit over a 2-week period. Subsequent changes were evaluated relative to the preoperation pooled mean. Statistical analysis was by Student's t-test using paired data where appropriate.

Plasma Samples.—Blood (2.5-3.0 ml) for determination of plasma renin activity, plasma renin substrate, and urea was collected from the marginal ear vein into cooled centrifuge tubes containing 30 units of heparin. The plasma was separated and stored frozen at −15°C until assay. Usually blood was collected only once a week, but on occasion, two samples were taken without a subsequent fall in hematocrit. A comparison of plasma renin activity and substrate levels using either heparin (10 units/ml blood) or ethylenediaminetetraacetic acid (EDTA) (5 mg/ml blood) as the anticoagulant failed to demonstrate any differences.

Blood Pressure.—Diastolic blood pressure in the conscious rabbit was measured twice a week by puncturing the dilated central ear artery with a half-inch 25-gauge needle connected through a low-displacement transducer (Statham P23Db) to an Offner dynograph. Flow in the artery was reduced by manual occlusion of the vessel distal to the puncture site during the recording of pressure. Measurements were made 30 minutes after the rabbit had been placed in a thermostatically controlled room (37°C). Under these conditions maximum dilatation of the artery was readily established by firm massage over the vessel.

A typical pressure tracing is shown in Figure 1: ear artery pressure obtained by the preceding method is compared with simultaneously recorded aortic pressure in a conscious hypertensive rabbit. Although the pulse pressure from the aortic catheter was twice that recorded from the ear artery, the diastolic pressures were identical. Damping in the recording system was not responsible for the lower pulse pressure in the ear artery because of the compliance in the peripheral arterial line. For this reason, diastolic pressure alone was used in all calculations.

Plasma Renin Activity.—Plasma renin activity (PRA) is a measure of the reaction rate between endogenous renin and endogenous substrate. PRA was determined on 0.5-1.0-ml samples of plasma by assaying the effects of the generated angiotensin on rat blood pressure according to the method of Skinner (17). The validity of this method for rabbit plasma was investigated as it was for human plasma (17), and it was confirmed that initial treatment at pH 4.5 in the presence of 0.005M EDTA completely suppressed angiotensinase activity with survival of both renin and substrate. Contrary to the findings in human plasma however, the rate of angiotensin formation was not linear in all plasma samples. In a preliminary study, 5 of 11 separate plasma samples incubated at pH 7.5 after initial treatment at pH 4.5 demonstrated a 20-40% slowing of the reaction rate over a 10-hour period with further slowing beyond this time. Since survival of angiotensin and substrate was complete during the incubation period and since consumption of substrate (less than 10%) could not account for the curved velocity, it was concluded that renin itself was being destroyed despite the presence of EDTA, Trasylol (FBA), and neomycin. To minimize this error, plasma was incubated for a fixed 6-hour period during PRA estimations. This length of time generated angiotensin levels in normal plasma (7-12 ng/ml hour−1) well above the threshold of the method (2 ng/ml hour−1).

Plasma Renin Substrate.—Plasma renin substrate (PRS) was measured in plasma after initial treatment by the PRA methodology using angiotensinase-free rabbit or human renin at a concentration sufficient to drive the reaction to completion in 10 minutes at 37°C without further change over 120 minutes. Renin substrate is expressed as released angiotensin II (µg/ml or moles/liter) measured by bioassay. Rabbit and human renin were prepared by trichloroacetic acid treatment of minced kidney (17). Human renin had no effect itself on rat blood pressure, but rabbit renin, which cross-reacts with rat substrate, was inactivated prior to substrate bioassay by heating for 5 minutes at 75°C. Rabbit and human renin yielded identical substrate levels from the same plasma sample.
Derived Plasma Renin Concentration.—Plasma renin concentration (PRC) was calculated from the Michaelis-Menton equation,

\[ V_{\text{max}} = \frac{v(1 + K_m/S)}{S} \]

or \( PRC = \frac{PRA}{(1 + K_m/PRS)} \),

using PRA and PRS measured for that plasma sample.

\( K_m \) for the rabbit renin-rabbit substrate reaction was averaged from the Woolf plots (18) shown in Figure 2 (\( K_m = 2.3 \mu g \) angiotensin II/ml or \( 2.3 \times 10^{-6} \)M). Substrate for this study was semipurified from plasma from rabbits which had been nephrectomized 4 days earlier. Single-column chromatography on DEAE-Sephadex with elution over a linear gradient from 0.02 to 0.5M NaCl at pH 7.0 in phosphate buffer was used for the purification. Substrate with a specific activity of 0.22 \( \mu g \) angiotensin/\( \mu g \) protein was eluted at 0.25–0.3M. The specific activity of the eluate was tenfold higher than that in the plasma from the nephrectomized rabbits and forty-fold higher than that in the plasma from normal rabbits. The eluate was concentrated by ultrafiltration to provide a substrate containing 10 \( \mu g \) angiotensin/ml with 45 mg protein/ml. The substrate was rendered angiotensinase-free with 0.001M EDTA and on incubation displayed no endogenous pressor or depressor activity.

Plasma Urea.—This estimation was performed by Laboratory Services Pty. Ltd., Melbourne, using an autoanalyzer method (Technicon).

Surgical Procedures.—Operations were performed under light ether anesthesia using aseptic precautions following medication with paraldehyde (1 mg/kg, ip) and atropine sulfate (0.3 mg/kg, iv). Unilateral nephrectomy was performed on 6 rabbits by removing the left kidney extraperitoneally through a flank incision. Bilateral renal wrap with cellophane (Dupont, no. 215PD) was performed in one operation in 7 rabbits as previously described (1). Single renal wrap with contralateral nephrectomy was performed in two operations in 21 rabbits. The left kidney was wrapped with cellophane during the first operation, and the right kidney was removed 7 days later. Decapsulation of the cellophane-wrapped kidney was performed in 10 rabbits made hypertensive by the single wrap-nephrectomy procedure. Decapsulation was accomplished by removal of the cellophane followed by incision and complete stripping of the thick fibrous capsule from the cortex. Blood loss was not significant. Renal mass was measured when the rabbits were killed (day 80–100) and after all extrarenal tissue including the renal capsule had been removed. Renal mass–body weight ratios were calculated using the last antemortem body weight.

Results

In Figure 3, blood pressure, PRA, PRS, PRC, and urea are shown for eight unoperated control rabbits. No parameter varied significantly during the 80-day period.

Unilateral Nephrectomy.—Figure 4 shows that nephrectomy had no effect on blood pressure. However, PRS was elevated at day 3 after surgery and did not return to near normal until day 7. The transient rise in PRS was accompanied by a slight but insignificant rise in PRA and by a fall in PRC. Return of PRS to normal was followed by slowly developing, prolonged falls in PRA and PRC which were statistically significant at day 11. By day 21
PRA and PRC had returned to normal. When the rabbits were killed at day 32, the average mass of the sole remaining kidney was 10.4 g compared with 8.0 g for single kidneys in control rabbits matched for breed, age, and weight. There was a significant rise in plasma urea after unilateral nephrectomy (Fig. 1).

**Bilateral Renal Wrap.**—Figure 5 shows that bilateral renal wrap resulted in a consistent and sustained hypertension. Blood pressure began to rise on day 14 and reached a plateau of 105 mm Hg on day 28. The only significant change in PRA, PRS, or PRC occurred early after surgery: PRS was markedly elevated and PRA and PRC were depressed. By day 7 PRS had returned to normal, and by day 14 PRA and PRC had also returned to normal. There was no significant change in plasma urea throughout the period.

**Single Renal Wrap with Contralateral Nephrectomy.**—Figure 6 shows that there was a transient rise in PRS and a transient fall in both PRA and PRC following wrapping of the left kidney. Prior to right nephrectomy on day 7, PRA and PRC were still depressed, but PRS had returned to normal. Following right nephrectomy, PRS was again transiently elevated, and there was a further significant fall in PRC. PRA and PRC returned to normal over a 14-day period following the nephrectomy. Although the wrapping procedure was not associated with any change in plasma urea, there was a significant rise in urea levels early after the right nephrectomy with a further rise later in the course of the hypertension.

**Decapsulation.**—The changes in rabbits subjected to decapsulation are shown in Figure 7. Following the wrap-nephrectomy procedure, the changes were similar to those illustrated in Figure 6. Hypertension developed; PRA and PRC were transiently suppressed but returned to normal prior to decapsulation on day 28. Decapsulation was associated with a transient elevation in PRS, and PRC remained significantly less than normal during the 14-day postdecapsulation period.

**Renal Mass and Body Weight.**—Postmortem renal mass of the rabbits made hypertensive by bilateral wrapping was greater than that of the controls, but the body weight of these rabbits was...
less than that of the controls. Thus, the renal mass-body weight ratio was considerably greater than normal (Table 1).

In the rabbits subjected to wrap, nephrectomy, and decapsulation (Fig. 7), the remaining kidney had hypertrophied; its mass at 80 days postdecapsulation was close to that for the two kidneys in control rabbits (Table 1). Renal mass data for the rabbits made hypertensive by the wrap-nephrectomy procedure (Fig. 6) was not recorded. However, in these rabbits it was noted at the postmortem examination that the wrapped kidney had hypertrophied in the region of the renal hilus where it had bulged through the cellophane wrapping and the thick fibrous capsule.

Discussion

The measurement of PRA, PRC, and PRS permits a more complete analysis of the renin-angiotensin system than can be obtained with any one value alone. PRC, as presently derived, yields information relating to renin secretion but cannot be used to imply anything about the physiological effect of the system. PRA measured at pH 7.5 relates more to physiological effect, since it incorporates the dependence of angiotensin production rate on substrate concentration. This dependence is known to hold for normal substrate levels in several species

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Since the concentration of renin substrate in plasma is only one fifth of the $K_m$ in the rabbit, alterations in substrate concentration might not be accompanied by analogous variations in PRA and PRC. Under such circumstances, each value provides different information about the in vivo operation of the system (19).

The present assay method was originally developed for human plasma but has already been applied to the rabbit by Romero et al. (22) in a study of hypertension induced by clipping the renal artery. In our hands, its application to rabbit plasma was complicated by a nonlinear formation of angiotensin that could not be accounted for by angiotensinase or substrate consumption and appeared to be due to loss of renin, probably by enzymatic degradation. This potential error was to a large extent obviated by incubating the samples for a short fixed period of time. The PRA values reported by Romero et al. (22) are less by half than the present ones; the difference could be related to the nonlinearity in our assay but could also be due to other factors such as sodium intake. Again, for PRS, our values were greater than those of Romero et al. (22), a difference that could be due to their use of hog renin, since Sen et al. (23) have claimed that hog renin generates less angiotensin from rabbit plasma than does homologous renin. Such a possibility does not, however, invalidate the directional changes described in their paper which are similar to the present findings.

The influence of substrate concentration on reaction velocity was defined in kinetic studies using semipurified rabbit substrate (Fig. 2). Normal PRA concentrations (0.5-0.7 μg/ml) lie at or near the first-order range, but because PRA often rises above this level it is more appropriate to derive PRC from PRA and PRS through the medium of the Michaelis-Menten equation than it is to assume as did Ryan et al. (24) that the reaction is first order under all circumstances. There has apparently been no previous attempt to estimate the $K_m$ value for the renin reaction in the rabbit.

The rate of onset of the hypertension produced by different cellophane wrapping procedures is pertinent to the changes in the renin-angiotensin system that might be expected in the present experiments. Bilateral wrapping caused a slow rise in pressure commencing after day 14 and reaching more moderate levels than those which developed with the single wrap-nephrectomy procedure. For the latter procedure pressure rose by day 2 following the unilateral nephrectomy. This finding supports the notion that mechanical compression of the renal parenchyma is the important factor leading to hypertension, since as Page et al. (25) pointed out compensatory hypertrophy within the fibrous hull is not as great with the bilateral procedure and compression is therefore less. In fact, our findings indicated that even bilateral wrapping provoked some renal hypertrophy. This observation is novel and suggests that perinephritis itself can interfere with the mechanism controlling renal mass. Protection of intrarenal arterioles from hypertensive disease has been shown by both Graef and Page (26) and Campbell and Santos-Buch (27) in perinephritic animals and is further evidence that compression sufficient to cause a pressure drop within the renal vascular system does occur. In this respect perinephritis may be viewed as being similar to the Goldblatt procedure.

When these considerations are related to the two renal mechanisms generally thought to influence arterial blood pressure, namely, the function relating output of salt and water to renal perfusion pressure and the function relating renal perfusion pressure to renin secretion (6), it would be expected that both mechanisms would be altered in a manner similar to that postulated for Goldblatt hypertension. In Goldblatt hypertension, when all
renal tissue is subject to hypoperfusion, both sodium retention and increased renin secretion occur at the onset of hypertension and each return to normal in the chronic phase when renal perfusion pressure beyond the constriction has risen to normal (10, 28, 29). Whereas acute renal parenchymal compression also causes immediate renin release (14), the slow compression of cellophane perinephritis might well provoke sodium retention more effectively than it produces renin release and actually lead to a fall in renin secretion.

A further consideration in interpreting the present results is the finding of Romero et al. (22) that following sham operations PRS is almost double control values at day 3 and returns to normal at some time before day 15. Presumably this change is mediated by adrenocorticotropic hormone (30). Since in their experiment the measured PRA was not changed on day 3, PRC and therefore renin secretion actually fell to nearly half the control value and returned to normal by day 15. The same rise in PRS and suppression of PRC was seen in our experiments following all surgical procedures. This effect was seen in its least complicated form following the bilateral wrap procedure illustrated in Figure 5 where no other influence was discernible. The effect was also seen following unilateral nephrectomy (Fig. 4), but in this case the initial rise in PRS and the initial fall in PRC were followed by a later fall in both PRA and PRC after PRS had returned to normal. This observation may indicate that, following the unilateral nephrectomy alone and before the compensatory hypertrophy, the reduction in renal mass leads to some fluid retention and suppression of renin secretion. Unilateral nephrectomy has not been previously studied with respect to renin levels in rabbits, but our findings are similar to those of Dauda et al. (31) in the rat. Taken together with the effect of operation alone on substrate levels, the sequence of changes in this normotensive group virtually accounted for all the changes seen following nephrectomy in rabbits subjected to the single wrap-nephrectomy procedure (Fig. 6). In these rabbits the onset of hypertension was associated with suppression of both PRA and PRC. Suppression in this group after nephrectomy was, however, less obvious, because both PRA and PRC were already less than normal due to the prior surgical procedure.

Decapsulation with subsequent fall in blood pressure was again associated with the nonspecific effect of surgery on PRS. Continued suppression of PRC and PRA following decapsulation might have facilitated the return of blood pressure toward normal and possibly reflected a continued slight overperfusion of these kidneys.

The results illustrate that suppression of renin secretion does not exclude the possibility that hypertension is of renal origin. Suppression of renin secretion during the evolution of perinephritis hypertension presumably means that a small and probably immeasurable increment in extracellular fluid volume overrides the direct renin-secreting tendency of renal compression (14). Such a conclusion concurs with many recent studies which have shown that chronic hypertensions in which all renal tissue is subject to similar manipulation are unlikely to be renin dependent exclusively. It cannot be concluded however that the renin-angiotensin system exerts no influence on the blood pressure in this or other forms of hypertension in which renin secretion is not increased. As in the normal animal and under all circumstances in the present hypertensive experiments, renin secretion (PRC) responds sensitively to altering circulatory conditions. The hypertensive influence of the system is then determined not only by the directional change in secretion but also by whether an appropriate degree of change has occurred with respect to all other simultaneously changing functions.

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References

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DUNCAN J. CAMPBELL, SANDFORD L. SKINNER and ALLAN J. DAY

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