Malignant Hypertension Resulting from Deoxycorticosterone Acetate and Salt Excess

ROLE OF RENIN AND SODIUM IN VASCULAR CHANGES

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ABSTRACT

The evolution of malignant hypertension was studied under metabolic balance conditions in 11 uninephrectomized rats given deoxycorticosterone acetate and 1% NaCl as drinking water. Changes in sodium and potassium balance were related to changes in blood pressure, plasma renin activity, hematocrit, and kidney histology. After 3-4 weeks of steadily positive sodium balance accompanied by continuously increasing blood pressure up to 185 ± 19 (SD) mm Hg, periods of sodium loss accompanied by evidence of hemoconcentration were observed marking the onset of the malignant phase as defined by the development of fibrinoid necrosis in the kidney. Plasma renin activity remained markedly suppressed both at the fourth week (0.33 ± 0.02 ng/ml hour⁻¹) when the sodium balance was positive and the kidney biopsy negative and at the end of the experiment (0.35 ± 0.36 ng/ml hour⁻¹) when the sodium balance was negative and the kidney histology revealed malignant vasculitis. Infusion of the angiotensin II inhibitor 1-Sar-8-Ala-angiotensin II consistently failed to affect blood pressure, and the kidney tissue norepinephrine level was reduced (0.054 ± 0.01 μg/g) compared with the control level (0.132 ± 0.02 μg/g). We conclude that malignant vasculitis in this model is preceded by hypertension associated with sodium and water retention and is accompanied by negative sodium balance, decreases in body weight, falling blood pressure, and hemoconcentration without demonstrable participation of the renin-angiotensin system or the renal catecholamines.

KEY WORDS

hemoconcentration angiotensin II inhibitor fibrinoid lesions
Wistar rats metabolic balance plasma renin activity

Malignant hypertension has classically been distinguished pathologically from benign essential hypertension by the presence of extensive fibrinoid necrosis of terminal arteries and arterioles as well as proliferative endarteritis. These changes have traditionally been considered to be the consequence of the mechanical stress of excessive filling pressure imposed on the overstimulated arterial muscular wall (1).

In human malignant hypertension, aldosterone secretion rates have often been found to be elevated (2). This observation has led to the finding that angiotensin controls aldosterone secretion (3) and to the hypothesis that renin is critically involved in the syndrome of malignant hypertension (4). This thesis has been supported by clinical observations (5) and by animal experiments in which a syndrome similar to human malignant hypertension has been reproduced by inducing high renin levels (6) or by administering exogenous renin or angiotensin (7, 8).

In contrast to this hypothesis that high renin levels are a prerequisite for malignant hypertension, there have been occasional reports of this disease in patients with excessive mineralocorticoid secretion but with presumed or measured low plasma renin activity (9, 10), although this latter situation is usually characterized by a relatively benign course. In addition, malignant changes have been produced in rats following unilateral nephrectomy and prolonged treatment with deoxycorticosterone acetate (DOCA) and 1% NaCl drinking water (11). Therefore, the question arises of whether induction of malignant hypertension is possible in the presence of low plasma renin levels or whether relative or absolute increases in plasma renin activity and angiotensin are a requirement for this process.

The present study was designed to investigate the role of changes in plasma renin activity in relation to changes in sodium, potassium, and...
blood pressure balance during a prolonged period of DOCA and 1% NaCl administration in uninephrectomized rats as the animals progressed from benign-phase to malignant-phase hypertension.

**Methods**

**GENERAL**

Male Wistar rats weighing 160-240 g were chosen for the present study. All of the rats underwent a left nephrectomy under ether anesthesia at least 7 days prior to the start of the experiments. Study rats were maintained on a synthetic diet like that described by Möhring et al. (12) and given 1% NaCl as drinking water. At weekly intervals DOCA (Percorten Pivalate, Ciba, 30 mg/kg body weight, sc) was injected.

**ANIMAL GROUPS**

**Group 1.**—Six rats were placed in individual metabolic cages following nephrectomy. After a 7-day period for adaptation to the metabolic cages and the synthetic diet, a 3-day control period of balance studies was carried out; blood samples were obtained for determination of plasma renin activity and hematocrit. After this control period, the tap water was replaced with 1% NaCl and the weekly injections of DOCA were begun. The rats were weighed daily, and 24-hour urine samples were collected. Balance studies for sodium and potassium were performed under conditions of constant temperature (24 ± 1°C) and humidity in a room with only natural light.

Systolic blood pressure was measured twice weekly. At the beginning of the fourth week, all of the rats were subjected to a renal biopsy, and venous blood samples for determination of plasma renin activity and hematocrit were taken for comparison with similar samples obtained during the postnephrectomy control period. The renal biopsy was performed via a lateral incision; the kidney was exteriorized and a small piece of cortex was removed using a razor blade (10-20 glomeruli/sample). Hemorrhage was stopped by slight pressure for 3 minutes, and the kidney was placed back in its previous position.

The study was continued for a period varying from 6.5 to 10 weeks depending on the condition of the individual rat. When the rats appeared moribund, blood was again taken for renin and hematocrit determinations, the rats were killed, and the heart, pancreas and kidney were obtained for histologic examination.

**Group 2.**—Five rats were followed in the same fashion as those in group 1 for determination of blood pressure, weight, plasma renin activity, and hematocrit, but metabolic balance studies were not performed.

**Group 3.**—Five of the uninephrectomized rats maintained on 1% NaCl and DOCA which appeared to have reached the malignant phase, as determined by the onset of weight loss and subsequently confirmed by pathologic changes, were selected for infusion with 1-Sar-8-Ala-angiotensin II, a competitive inhibitor of angiotensin II (13). Under ether anesthesia, the femoral vein (PE 10 catheter) and the external iliac artery (PE 20 catheter) were cannulated. Arterial blood pressure was monitored with a Statham strain gauge. On awakening, the rats were maintained in a semirestrained position. After a 30-minute control period, the angiotensin inhibitor was infused at a rate of 9 ng/min for 60 minutes. Inhibition was confirmed by the failure of the blood pressure response to an injection of 200 ng of angiotensin II. At the end of the infusion, an additional bolus of 300 ng of the inhibitor was given. At the termination of the study, organs were obtained for histologic observation.

**Group 4.**—Kidney tissue levels of norepinephrine were measured in four experimental DOCA-treated rats and four uninephrectomized control rats at the fourth week and in five experimental rats and five control rats between the sixth and the eighth week when the DOCA-treated rats were reaching the malignant phase, as later confirmed by pathologic study. In these rats, the kidney was obtained following decapitation and weighed; a portion was saved for pathologic examination, and the remainder was frozen for tissue norepinephrine assay.

**ANALYTICAL METHODS**

**Sodium and Potassium Balance.**—Sodium and potassium balance studies were carried out in metabolic cages (Acme Research Products). The 24-hour urine volume was measured, and the collecting funnels were washed with 500 ml of demineralized water. The sodium and potassium in the combined collection was measured with a flame photometer and corrected to 24-hour excretion. The dried feces for each 24-hour period were wet ashed with 2 ml of concentrated HNO_3 for 18 hours and diluted to 10 ml for sodium and potassium determinations. The intake of 1% NaCl was measured daily, and a daily sample of the drinking water was analyzed for sodium. The daily food intake, of known sodium and potassium content, was also measured. Therefore, the sodium and potassium intake could be calculated on the basis of the daily food and drinking water consumption. The sum of urinary and fecal sodium and potassium excretion per 24-hour period was taken as the total excretion. These calculations then allowed the determination of sodium and potassium balance.

**Systolic Blood Pressure.**—Systolic blood pressure was measured in unanesthetized rats by the tail microphone method.

**Plasma Renin Activity.**—Samples of 1.0 ml of blood for determination of plasma renin activity (ng/ml hour⁻¹) were taken under light ether anesthesia from the jugular vein with a regular 2-ml syringe fitted with a 21-gauge needle after the uninephrectomy, before DOCA and NaCl administration, after 4 weeks of DOCA treatment, and prior to death. The plasma renin activity was measured in 0.2 ml of nonhemolyzed plasma by the radioimmunoassay of generated angiotensin I as described by Sealey et al. (14).

**Hematocrit.**—Blood was collected in heparinized capillary tubes, and the hematocrit was determined by a micromethod. A peripheral blood smear was stained for red cell morphology.

**Kidney Norepinephrine.**—For analysis of kidney norepinephrine, the rats were killed by decapitation, and their kidneys were removed immediately, quickly freed of their capsules, washed in normal saline, lightly blotted, and weighed. A small specimen for histologic studies was taken from each kidney, and the rest was frozen instantly in Dry Ice and stored at −70°C for less than 2 weeks. Prior to assay the kidneys were homogenized in three volumes of perchloric acid (0.4M, final
concentration) and centrifuged at 30,000 g for 15 minutes. Norepinephrine was extracted from the supernatant fluid on alumina by the method of Anton and Sayre (15) as modified by Neff and Costa (16). Norepinephrine was assayed by the fluorometric procedure of Chang (17) and read in an Aminco-Bowman spectrophotofluorometer. Internal standards and reverse oxidation tissue blanks were included for each assayed kidney. The data are presented as µg norepinephrine/g wet weight.

PATHOLOGIC EXAMINATION

Renal tissue obtained at biopsy and autopsy for histologic examination was fixed in buffered Formalin solution, and sections were taken for routine (hematoxylin, phloxin, safran [HIPS] and fibrin [Martius scarlet blue]) staining. In addition to the kidney, histologic observations were made on tissue obtained from the pancreas and the heart. All histologic material was examined without knowledge of group or treatment.

The presence of acellular material staining as fibrin (fibrinoid) within the blood vessel wall or within the lumen (luminal fibrinoid) with or without necrosis of the blood vessel wall was characterized as positive histology for malignant changes.

Results

REPRESENTATIVE STUDY

Figure 1 demonstrates the evolution of the alterations observed in an individual rat over the entire period of DOCA and 1% NaCl administration. The initial 3-week benign phase was characterized by a progressive rise in blood pressure, a stable hematocrit, positive sodium balance, weight gain, overall negative potassium balance, and suppression of plasma renin activity. Beginning at the sixth week, a period of negative sodium balance associated with weight loss occurred. This period was followed by 1 week of positive sodium balance and weight gain; again at the end of this week, the sodium balance became consistently negative, the rat lost weight, and the blood pressure fell. During the terminal period, there was a dramatic rise in hematocrit associated with a negative sodium balance and weight loss, and the kidney pathology revealed fibrinoid changes despite the fact that there was no elevation of the plasma renin activity in response to the sodium loss.

BLOOD PRESSURE

Elevation of blood pressure was present at the beginning of the third week, and all of the rats were hypertensive at the time of renal biopsy at the beginning of the fourth week (186.7 ± 19.0 mm Hg) (Fig. 2). However, there was no significant rise in blood pressure after the fourth week, and a...
slight fall in blood pressure (175.0 ± 18.0 mm Hg) occurred during the final period of weight loss, negative sodium balance, and clinical deterioration.

SODIUM AND POTASSIUM BALANCE

Cumulative weekly balance data for sodium and potassium in the six rats maintained in the metabolic cages are shown in Figure 3. During the first 3 weeks of DOCA and 1% NaCl administration, the sodium balance was consistently positive and the DOCA escape phenomenon was not observed; the potassium balance was generally negative. Thus, at the time of the renal biopsy and up to the fourth week, all of the rats had only positive weekly sodium balances. In contrast, beginning as early as the fourth week in some rats, periods of negative sodium balance appeared which alternated with periods of positive balance. Generally, two to three oscillations occurred, and in five of six rats the final moribund condition occurred during a period of negative sodium balance. In the other rat, the last period of negative balance was followed by a final 18 hours of positive balance which occurred in conjunction with acute pulmonary edema and ascites—findings not present in the other rats. The potassium balance continued to remain negative with occasional periods of positive balance.

As shown in Figure 4, the changes in sodium balance closely correlated with the weekly changes in body weight (correlation coefficient $r = 0.9390$, $P < 0.001$).

Despite the periods of negative sodium balance that occurred after the third week, the total cumulative balance was positive in all of the rats as expected for growing animals being given mineralocorticoid (Table 1). In contrast, the total potassium balance was negative in five of six rats, and in the one rat with a slightly positive potassium balance this potassium gain was clearly less than that expected in normal growing rats (12). This negative balance can be attributed to the mineralocorticoid excess.

HEMATOCRIT

The hematocrit did not change significantly from the control period to the fourth week of DOCA.
Correlation of weekly cumulative sodium balance and weekly changes in body weight. Solid squares = rat 1, open circles = rat 2, open squares = rat 3, solid triangles = rat 4, solid circles = rat 5, and open triangles = rat 6.

HISTOLOGIC FINDINGS

All kidney biopsies taken during the control period and at the beginning of the fourth week, when the rats had established hypertension, were negative for fibrinoid depositions or necrosis of the vascular wall. In contrast, the final pathologic examination, during the period of weight loss and negative sodium balance, was positive for fibrinoid depositions, with or without necrosis of the vascular wall, in all of the rats. In addition, 3 of 11 rats demonstrated tubular necrosis. Moreover, fibrinoid changes were found in the heart of 9 of 11 rats and in the pancreas of all of the rats. The hearts of all the DOCA-treated rats had scars, but none of the uninephrectomized controls had either myocardial scars or fibrinoid lesions.

PLASMA RENIN ACTIVITY

The plasma renin activity during the control period and the two experimental periods in all 11 rats is shown in Figure 5. In the control period, the plasma renin activity was 20.79 ± 4.46 (sp) ng/ml hour⁻¹. At the fourth week there was a dramatic and significant (P < 0.01) reduction in renin to 0.33 ± 0.02 ng/ml hour⁻¹. Furthermore, during
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Plasma renin activity during control, benign, and malignant phases. Plasma renin activity was constantly suppressed throughout the experiment in comparison with the control period.

the final period of malignant changes with negative salt balances and weight loss, the renin level did not rise but remained low at a final value of 0.35 ± 0.36 ng/ml hour⁻¹.

Angiotensin Blockade

The infusion of the competitive inhibitor of angiotensin II did not produce a drop in blood pressure in any of the five rats studied (175.70 ± 4.3 mm Hg at 15 minutes and 177.3 ± 3.95 mm Hg at 30 minutes of infusion compared with 171.4 ± 4.8 mm Hg before the infusion) (Fig. 6). All of these rats were subsequently shown to have arteriolar changes characteristic of malignant hypertension on pathologic study. There was actually a slight, although insignificant, rise in blood pressure during the first 10-15 minutes of infusion. Thus, the circulating angiotensin level, presumably low as shown in the other rats, did not play any role in the maintenance of the hypertension in these rats with malignant hypertension.

Kidney Norepinephrine Levels

The kidney norepinephrine level (Fig. 7) was significantly lower (P < 0.01) than that found in the matched controls at both periods of determination. At the fourth week (benign phase), the mean value in the DOCA-treated rats was 0.042 ± 0.01 µg/g. In the malignant DOCA-treated rats (6-8 weeks), the mean level was 0.054 ± 0.01 µg/g; the level was 0.132 ± 0.02 µg/g in the matched controls. Thus, there was no significant rise in the tissue content of kidney norepinephrine when the DOCA-treated rats became malignant; the values remained significantly lower than those found in the controls.

Discussion

In this study malignant hypertension was induced in rats by administration of deoxycorticos- terone acetate and 1% saline. The histologic changes characteristic of malignant vasculitis, i.e., fibrinoid necrosis of the arterioles, were present in every rat. These findings are in keeping with the observations made by Selye in 1943 (11).

Traditionally, hypertension has been considered to cause these changes by mechanical stress related primarily to the degree of pressure elevation. In the present experiments, however, the degree of blood pressure elevation, although undoubtedly important, did not seem to be exclusively responsible for the induction of the malignant changes. Thus, vascular damage developed in the phase of falling blood pressure, whereas no histologic signs of malignant vascular changes were observed when blood pressure was highest at the fourth week. It could be argued at this point that the duration of hypertension was not sufficient to produce vascular changes at the fourth week, although it did so in another 2-6 weeks, and that it was this longer-lasting mechanical effect rather than other factors such as the paroxysms of natriuresis which is the major causative factor.

However, different experimental models argue against this idea. Thus, animals with equally high or even higher levels of blood pressure sustained through similar periods, such as rats with established hypertension of renal origin, do not usually develop such extensive malignant vascular changes even though DOCA-treated rats with low plasma renin activity do in shorter periods of time.
Kidney tissue norepinephrine level in rats treated with DOCA and NaCl was constantly lower at both the benign and the malignant phase in comparison with that in uninephrectomized control rats tested over the same period of time.

(18). Evidence from other investigators has also suggested that enhanced mechanical stress on the vascular wall resulting from hypertension, although very important, is not the only causative factor responsible for the development of vascular damage (19).

Another factor that has been thought to be critically involved in the causation of vascular damage of hypertension is the pressor hormone angiotensin II. However, in the present experiment, plasma renin activity, the main determinant of angiotensin II generation, was shown to be suppressed to nearly undetectable levels at the time when the malignant vascular changes developed. One could argue that even minute and undetectable increases in renin, still within the subnormal range, could have been involved in inducing the malignant phase, in view of the greatly increased sensitivity of DOCA-treated animals to angiotensin (20). However, against this line of reasoning was the absence of any fall in blood pressure during the specific blockade of angiotensin II by infusion of the angiotensin II inhibitor during the malignant phase, indicating that this small amount of renin did not contribute to the maintenance of hypertension.

Norepinephrine, another powerful vasoconstrictor substance, has been used to induce gross vascular changes in animals (21). We therefore measured the kidney tissue levels of norepinephrine during both the benign and the malignant phase of DOCA-induced hypertension. The norepinephrine levels were low in comparison with those in untreated uninephrectomized control rats during both these periods. This finding provides indirect evidence that the production of malignant vasculitis in the kidney was not initiated by a local increase in catecholamines.

In the absence of positive evidence for a role for vasoconstrictor substances in the pathogenesis of vascular damage, it seems worth pursuing the view that the mineralocorticoid-induced salt excess was in itself sufficient to account for the changes observed. Indeed, similar vascular damage has been induced in rats by the administration of excessive amounts of salt (22). Less salt is needed to produce the same changes when the renal mass is reduced prior to the study (23). Thus, it appears that both reduced renal mass and DOCA greatly increase the susceptibility to salt. DOCA administration alone or a reduction in renal mass alone, without sodium loading, does not produce these changes (11). Taken altogether, these observations suggest that the development of malignant changes in these models could ultimately be a consequence of altered sodium metabolism.

In the present study, the DOCA escape phenomenon was not observed as an intercurrent phenomenon, since sodium accumulation occurred until the fourth week. Presumably, salt and consequently fluid retention caused a gradual expansion of blood volume and an increase in blood pressure. The absence of vascular changes as observed in renal biopsies at this stage characterizes this phase of development of hypertension as benign. Following this period, two to four periods of natriuresis alternating with periods of sodium retention occurred, and the sodium loss was associated with a concomitant weight reduction. It is noteworthy that similar episodes of natriuresis have been observed to coincide with the onset of malignant hypertension in two-kidney Goldblatt rats, which is the typical model of renin-dependent hypertension (24). Death occurred during a period of negative sodium balance. In only one rat was this terminal period of negative sodium balance interrupted by a few hours of sodium retention associated with massive ascites and acute heart failure with pulmonary edema. These sudden losses of fluid resulted in hemoconcentration as evidenced by significant increases in hematocrit, observed in all rats who did not develop anemia. Those rats who became anemic exhibited the characteristic red cell fragmentation in peripheral blood smears, indicating the presence of microangiopathic hemolysis (25). Renal histology at this stage revealed widespread vascular damage characteristic of malignant vasculitis in all of the rats.

Thus, these observations demonstrate that the development of malignant hypertension was close-
ly related to the occurrence of periods of negative sodium balance which were associated with marked and sudden decreases in body weight, with hemoconcentration, and with a tendency for blood pressure to fall (Fig. 1). An alternative explanation could be that these episodes represent the result rather than the cause of malignant vasculitis. However, if occlusion, mechanical obstruction, and necrosis of arterioles and glomeruli were the primary changes, one would expect the resulting reduction of actively perfused renal mass to cause progressive renal failure with continuous accumulation of salt rather than episodes of uncontrollable natriuresis alternating with periods of sodium retention.

These findings enable us to hypothesize as to the mechanism of sodium-mediated vascular injury. In rats with DOCA-induced hypertension, it is known that the subendothelial tissues of the arteries and arterioles are rich in mucopolysaccharides, water, and sodium. The accumulation of these elements may tend to produce swelling of the vascular wall possibly resulting in a reduction of the lumen (26-28). This swelling should reduce the capacity of the vascular bed and consequently tend to decrease the blood volume. In support of this hypothesis is the slight increase in hematocrit observed in all of the rats in the fourth week, at a time when one might expect a reduction in hematocrit due to overexpansion of the blood volume by salt retention. As a result of the vascular wall edema, nutrition of the small arterioles may be compromised.

For each period of sudden natriuresis with fluid loss occurring as a consequence of critically increased blood pressure (29), the resulting hemoconcentration initiates further deterioration of vascular wall nutrition via two simultaneously occurring mechanisms: Due to fluid loss, plasma volume is decreased and blood viscosity enhanced, both of which tend to decrease blood flow and impair tissue perfusion. Hemoconcentration also enhances the process of intravascular coagulation with fibrin deposition at the luminal wall. These effects in the presence of an already edematous vascular wall lead to ischemia of the arteriolar wall, local tissue degeneration, or even necrosis of the subendothelial tissues of the arteries and arterioles.
lial tissues due to impairment of nutrition which is known to be totally dependent on normal intraluminal flow. The coexistence of elevated blood pressure with already initiated arteriolar wall damage will further enhance plasmatic vasculosis (30) with fibrin deposition in the subendothelial tissues resulting in fibrinoid necrosis of the vessel and at a later stage hyalinization (31).

In this pathogenetic sequence in which each episode is apparently initiated by natriuresis and resultant hemoconcentration, the kidneys are especially vulnerable. Increasing numbers of nephrons are damaged or destroyed by fibrinoid lesions, perhaps accentuated in the renal cortical vasculature by the effect of plasma skimming (32). Each recovery phase is associated with some healing of the lesion, reinstitution of positive sodium balance and restoration of blood volume consequent to increased thirst. Thus, the animal limits the phase of hemoconcentration and disseminated coagulation. But with another reexpansion of blood volume and the ensuing rise in blood pressure the next paroxysm is initiated.

Ultimately, progressive renal damage renders the kidneys unable to cope with further salt and fluid load, and death can result from vascular changes in the heart or brain, from microangiopathic hemolytic anemia (25), renal failure, or congestive cardiac failure. Figure 8 illustrates the proposed hypothetical sequence, which represents a different, admittedly still speculative, approach to the mechanism of hypertensive vasculitis in this model.

Do these findings have any relevance for the understanding and management of clinical hypertension? It is known that vascular damage in clinical hypertension, i.e., malignant hypertension, is very often associated with high renin levels (5, 33). However, several investigators have described low or normal renin levels in a certain fraction of their patients with malignant hypertension (33, 34). Furthermore, there are in the literature two possible cases of primary aldosteronism which developed malignant vascular changes (9, 10). These cases may be clinical counterparts of the experiment described in the present paper, in which sodium excess appears to be the main agent responsible for the vascular lesion.

The theory of sudden natriuresis and hemoconcentration as a factor initiating malignant vascular damage may be supported by some other observations which also suggest therapeutic approaches. Thus, it has been shown that saline administration may reverse malignant experimental renal hypertension (24) and that sodium depletion with diuretics may induce vascular damage (35). Observations supporting this possibility have also been made in toxemias of pregnancy (36, 37). Accordingly, in certain clinical settings already associated with hemoconcentration and vasoconstriction, diuretic therapy may be contraindicated, and in some cases it is even possible that saline administration may be beneficial.

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