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Production of Renal Hypertension in Adrenalectomized Dogs on Constant Hormone Replacement Therapy (40003)

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The onset of renovascular hypertension occurs concurrently with transient activation of the renin-angiotensin system (1-3). Angiotensin II (AII) directly stimulates aldosterone secretion from the adrenal cortex (4-7). Indeed, plasma aldosterone levels and aldosterone secretion rates increase during the acute high renin phase of renovascular hypertension (3, 8, 9). Thus, it is possible that genesis of this pathophysiological condition is dependent on mineralocorticoid hypersecretion.

The present studies were designed to evaluate whether excess mineralocorticoid activity resulting from activation of the renin-angiotensin-aldosterone system is essential for production of sustained renovascular hypertension. For this study an experimental model was used in which increased angiotensin II levels characteristic of the acute high renin phase of renal hypertension could not exert a direct steroidogenic effect on the adrenal cortex. In this regard, the renal artery was constricted in one kidney, adrenalectomized dogs receiving constant exogenous steroid maintenance therapy.

Materials and Methods. Calm female hounds (14.5 to 25.0 kg) were anesthetized with sodium pentobarbital and laparotomized. A unilateral nephrectomy was then performed after verifying the existence of a single renal artery leading to the remaining kidney. Chronic femoral arterial and venous catheters were passed subcutaneously and exteriorized between the shoulder blades in most of these dogs. The dogs were allowed to recover for 7 days after surgery before beginning several days of control data collection. They were fed a daily diet containing 60-65 mequiv of sodium, and water was available *ad libitum*. Urine collections were made for determination of daily urinary output and sodium balance. Arterial pressure was measured from the femoral arterial catheters in conscious animals. Arterial blood samples were also collected and centrifuged for plasma separation and bioassay of plasma renin activity (PRA). Plasma electrolytes were determined by flame photometry and blood hematocrits were measured. In the four dogs without chronic catheters, blood pressure was measured by direct percutaneous femoral artery puncture under local anesthesia, and blood samples were obtained from the jugular vein.

One-kidney renovascular hypertension was produced in seven dogs. The renal artery of the kidney was exposed retroperitoneally. After bathing the area with 2% lidocaine solution, an adjustable constricting device and an electromagnetic flow probe were placed around the renal artery. After allowing 10-20 min for stabilization of blood flow, the renal artery was constricted to reduce renal blood flow by 56 ± 2%. After renal artery constriction, data were obtained daily for 7 days and, thereafter, three times each week for at least 1 month; chronic sodium balance studies were also conducted. The dogs were force fed when necessary to assure constant sodium intake. Prior to and at weekly intervals after renal artery constriction, plasma volume was also estimated by T-1824 dye dilution.

An additional group of eight dogs underwent bilateral adrenalectomy when the unilateral nephrectomy was performed. These dogs received pretreatment with 100 mg of cortisone acetate im the day before surgery. On the morning of the day of surgery, they were given 100 mg of cortisone orally, and an additional 50 mg of cortisone im immediately before adrenalectomy. Thereafter, the dogs were maintained on daily im ste-

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roid therapy of 20 mg of cortisone acetate and 2 mg of deoxycorticosterone acetate (DOCA), and data were collected immediately before steroid therapy, steroids were given as a single dose at 9:30 AM. The renal artery was constricted 1 week after adrenalectomy. The first four animals of this group were given a supplemental dose of 100 mg of cortisone acetate im immediately after renal artery constriction. Since these animals recovered readily, the remaining four adrenalectomized dogs received no additional cortisone during the surgery to constrict the renal artery. Observations were made in the same manner as for the group of dogs with intact adrenals. Completeness of adrenalectomy was verified by failure to measure discernible levels of plasma aldosterone by a radioimmunological technique (7).

Data from all experiments were analyzed by use of the Student's paired t test or by Student's t test for group comparisons, critical to a 5% significance level.

Results. Renal artery constriction in dogs with intact adrenals. Constriction of the renal artery in the seven dogs having intact adrenals produced a prompt increase in arterial pressure in all but one animal. The adjustable clip was later retightened in this dog and renal hypertension occurred. Mean arterial pressure increased during the first day after renal artery constriction in all dogs ($P < 0.05$) and reached a steady level of elevation of approximately 20 mm Hg by the second or third day after constriction (Fig. 1). Studies were terminated in two dogs within 2 weeks after renal artery constriction because they developed malignant hypertension.

In dogs with intact adrenals, PRA increased transiently ($P < 0.05$) for the first 6 days after renal artery constriction and then returned to control levels (Fig. 2). Cummulative sodium balance data for this group showed that sodium retention of about 75 mequiv occurred during the first 2–5 days after renal artery stenosis, but the dogs excreted most of this excess salt load by 6–10 days after renal artery constriction. Thereafter, they generally remained in normal sodium balance, although chronically there may have been a tendency toward

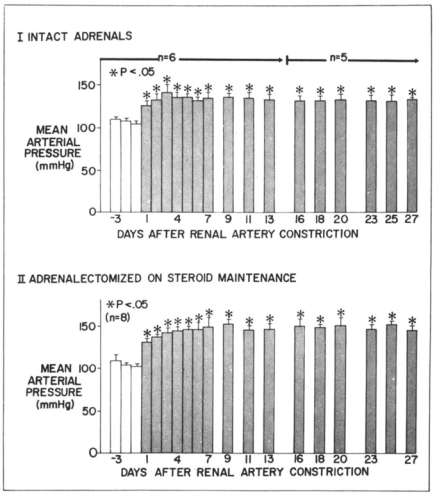

FIG. 1. Changes in mean arterial pressure after renal artery constriction in dogs with intact adrenals and in adrenalectomized dogs on constant steroid maintenance of cortisone acetate (20 mg/day) and DOCA (2 mg/day). Asterisks denote differences significant at 5% level from corresponding preconstriction control values, and shaded bars indicate values after renal artery constriction. Data appear as the mean ± SEM.

slight sodium retention. However, there was no detectable expansion of plasma volume during the 4 weeks after renal artery constriction. Hematocrit decreased from 36 ± 2 to 28 ± 2 ($P < 0.05$) during the first 6 days after renal artery constriction and then returned toward control levels.

Renal artery constriction in adrenalectomized dogs. Hypertension occurred following the initial constriction of the renal artery in six of the eight adrenalectomized dogs. Reconstricting the renal artery successfully produced sustained hypertension in the other two dogs. Apparently, hypertension was equally well produced in adrenalectomized dogs whether or not they received supplemental cortisone acetate therapy immediately following constriction of the renal artery. Also, since supplemental cortisone did not in itself affect any other variables that were measured, the data were pooled for all eight of these adrenalectomized dogs.

Mean arterial pressure rose promptly after renal artery constriction ($P < 0.05$) (Fig. 1), and the response was not different from that observed in dogs with intact adrenals. The adrenalectomized animals also

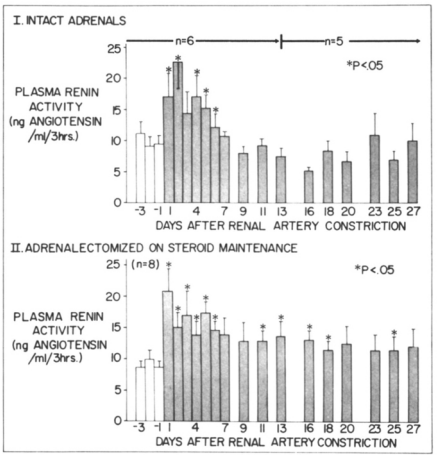

FIG. 2. Changes in plasma renin activity after renal artery constriction in dogs with intact adrenals and in adrenalectomized dogs on constant steroid maintenance of cortisone acetate (20 mg/day) and DOCA (2 mg/day). Asterisks denote differences significant at 5% level from corresponding preconstriction control values, and shaded bars indicate values after renal artery constriction. Data appear as the mean ±SEM.

showed a transient decrease in hematocrit after constricting the renal artery, similar to that which occurred in intact dogs. Further, they exhibited a marked increase in PRA for 6 days of approximately the same magnitude as occurred in the dogs with intact adrenals (Fig. 2). However, PRA levels remained somewhat elevated throughout the observation period in the adrenalectomized group, a response different from that of the dogs with intact adrenals. Renal artery constriction produced virtually the same pattern of changes in cumulative sodium balance in the adrenalectomized dogs as occurred in the dogs with intact adrenals with one exception: Sodium balance was slightly positive during the last week in the adrenalectomized dogs. However, after production of renal hypertension in the adrenalectomized dogs, plasma volume increased from a control value of 1040 ± 90 to 1205 ± 50, 1165 ± 35, and 1335 ± 105 ml for the next 3 weeks ($P < 0.05$ for all three values), so the additional sodium retention during the fourth week did not manifest itself by an increase in plasma volume.

The results also show that the daily steroid maintenance of 2 mg of DOCA and 20 mg of cortisone acetate was adequate to maintain normal plasma electrolyte concentrations. In dogs with intact adrenals, control plasma sodium and potassium concentrations were 143 ± 5 and 4.3 ± 0.1 mequiv/liter, respectively, while corresponding values in adrenalectomized dogs before renal artery constriction were 144 ± 1 and 4.2 ± 0.2 mequiv/liter. Production of renal hypertension did not alter these plasma electrolyte levels in either group.

Discussion. Early investigations on the role of the adrenal cortex in the genesis of renal hypertension were conducted 40 years ago (10, 11). Much of the existing literature suggests that induction of renal hypertension requires adequate adrenocortical activation. However, it has been uncertain whether the general inability to produce or sustain renal hypertension in nonmaintained, adrenalectomized animals is due to a nonspecific effect of adrenal origin (12–14). A possible adrenal factor which has been considered necessary for induction of renal hypertension is mineralocorticoid hypersecretion. This is an attractive possibility since the renin–angiotensin–aldosterone system is known to be activated during the early phase of renal hypertension (3, 8). In fact, Atwill *et al.* (15) reported that renal hypertension could not be induced or maintained in adrenalectomized dogs receiving only glucocorticoid replacement therapy. Also, it has been reported that spironolactone, a competitive inhibitor of aldosterone, reverses chronic renal hypertension in the rabbit (16). On the other hand, there is considerable evidence that aldosterone hypersecretion may not be necessary. Renal hypertension was produced despite administration of an aldosterone antagonist (17) and was maintained in adrenalectomized sheep for 48 hr after withdrawal of steroid therapy (18).

The purpose of the present study was to determine if chronic hypertension would develop in adrenalectomized dogs following renal artery constriction during maintenance on constant steroid replacement therapy. Under these circumstances the animals were never subjected to a high plasma level of aldosterone or mineralocorticoid excess for even a brief period. The experiment was done to exclude the possibility that

mineralocorticoid excess for a short time might trigger a pathogenic mechanism important for the development of chronic hypertension. Available data have never answered the question of the necessity of early activation of the renin-angiotensin-aldosterone system for chronic hypertension to occur. The present results were clearly negative; chronic hypertension developed and was sustained for 27 days during a constant low dose of 2 mg/day of DOCA along with 20 mg of cortisone daily. This dose was selected because in our past experience (19) these levels of replacement therapy are no more than enough to keep adrenalectomized dogs healthy.

The results of the current study agree with the already well-documented literature concerning the pathogenesis of one-kidney renovascular hypertension. Specifically, the renin-angiotensin system is activated during the first few days in this form of hypertension. It has been suggested (1, 2) that this early phase is largely renin dependent, but chronic one-kidney hypertension is clearly renin independent; the present dogs with intact adrenals had normal values for PRA during chronic hypertension. Also, the cumulative sodium balance data do not provide evidence for further extracellular fluid volume expansion during the chronic phase of hypertension. Our findings support recent reports (20, 21) that one-kidney renal hypertension is sustained by factors other than hypervolemia per se.

In contrast to the dogs with intact adrenals, the chronic adrenalectomized hypertensive dogs had a significant elevation in PRA. This finding indicates that the renin-angiotensin system might contribute to the chronic maintenance of hypertension in this adrenalectomized model, as has been reported previously (22). The stimulus for the chronic elevation of PRA in these adrenalectomized hypertensive dogs is unknown. These animals were not in negative sodium balance during the last 2 weeks; instead sodium balance was slightly positive during the last week. Also, plasma volume was increased and plasma electrolytes were normal, so there is no obvious explanation for the increase in renin release.

Summary. This study assessed the importance of increased aldosterone secretion secondary to acute activation of the renin-angiotensin system for development of chronic one-kidney renal hypertension. In dogs with intact adrenals, reduction of renal blood flow by 55-60% promptly produced mild chronic hypertension. The onset of hypertension was associated with transient sodium retention and hyperreninemia, but plasma volume did not change. Although mineralocorticoid hypersecretion was impossible in adrenalectomized dogs maintained with constant steroid therapy, hypertension was still successfully produced. In contrast to the dogs with intact adrenals, hypertension in the adrenalectomized group was characterized by chronically increased plasma renin activity and plasma volume. The results indicate that hypersecretion of mineralocorticoid hormones during the early high renin phase is not essential for production of chronic one-kidney renal hypertension.

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