Renal Hypertension and Renin Hypertension in Intact and in Adrenalectomized Rats (34151)

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(Introduced by Alvin P. Shapiro)

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The literature on the role of the adrenal gland in renal hypertension in the rat is controversial. Freed and St. George (1) described the blood pressure falling to normotensive levels following adrenalectomy in renal hypertensive rats, although the rats were maintained on 0.9% saline postoperatively. They also found that the administration of prednisolone restored blood pressure to the original hypertensive level.

Yet, both the development of renal hypertension in adrenalectomized rats maintained on 0.9% saline (2) and the maintenance of an established renal hypertension in similarly treated rats (3) have been reported. On the other hand, D,L-aldosterone or cortisol were ineffective in maintaining renal hypertension in adrenalectomized rats given water to drink (4, 5), whereas deoxycorticosterone (Doca) did (5). However, it was reported later (6) from the same laboratory that development of renal hypertension was prevented by adrenalectomy, although the rats were injected with Doca.

Masson and co-workers showed that in the rat, repeated subcutaneous injections of renin induced hypertension which closely mimics (7–10) the renal hypertension which develops after unilateral renal artery constriction. They found that renin-induced hypertension did not occur in adrenalectomized rats maintained on 1% saline (11). Cortisol restored this hypertension whereas Doca was ineffective (11).

Because of the confusion in the literature, the present experiments were performed to compare the role of the adrenal gland in renal hypertension and renin-induced hypertension in rats. In addition, the effect of various corticosteroids on the development of renin-induced hypertension in adrenalectomized rats was studied.

Materials and Methods. Male rats of a Wistar strain bred in this laboratory and weighing 120–140 g were used. Operations were performed under ether anesthesia. The adrenal glands were removed by lateral flank incisions. In all studies the adrenalectomized rats were given 0.9% saline to drink. Renal hypertension was induced by the application of a clip to the left renal artery (12).

Systolic blood pressure of unanesthetized trained rats was determined daily using a tail sphygmographic method as described previously (12). The investigator who performed the blood pressure measurements had no prior information about the kind of treatment which the rats received.

Plasma renin activity was measured according to the method of Pickens *et al.* (13), modified as described previously (14).

The partially purified renin used in these experiments was prepared from rat kidneys. A modification of the procedure of Haas *et al.* (15) was used. All steps were done at 0–5°, unless otherwise stated. Cleaned whole kidneys (generally 1 kg) were homogenized in 1 vol of water for 7 min in a Waring Blendor. The homogenate was subjected to ultrasonic disintegration (Branson, Sonifier) at 20 kcps for 2 min at maximum power in a cooling bath, followed by constant stirring for 1.5 hr with a magnetic stirrer at room temperature. By this procedure the total yield of renin increased 2–3-fold. Subsequently the mixture was centrifuged in a preparative ultracentrifuge (International-B 35), at 75,000*g* (av) for 20 min. The precipitate was

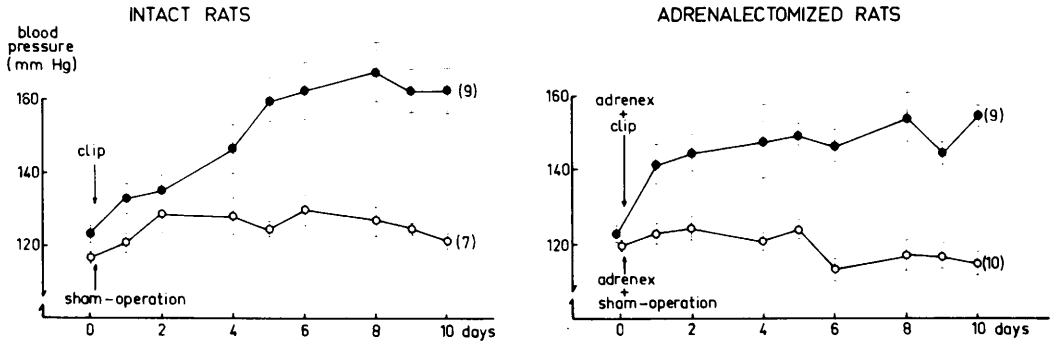

FIG. 1. Development of renal hypertension in intact and in adrenalectomized rats: (●), rats with renal arterial constriction; (○,●), mean values; bars indicate standard error of the mean; number of rats given in parentheses.

re-extracted with 1 vol of water. The combined supernatants were adjusted to pH 2.6 with 5 *N* H_2SO_4, stirred for 10 min, readjusted to pH 7.0 with 2.5 *N* KOH and then centrifuged at 800*g* (av) for 60 min. The supernatant was adjusted to pH 4.5 with 2.5 *N* H_2SO_4 and ammonium sulfate was added to a final concentration of 2.2 *M*. This mixture was stirred for 15 min, centrifuged at 800*g* (av) for 60 min and the precipitate was dissolved in 10 vol of water. This suspension was dialyzed twice against 50 vol of water for 24 hr. The retentate was centrifuged at 20,000*g* (av) for 10 min, washed with water once and the combined supernatants lyophilized. One kg of fresh tissue yielded 300–550 mg of partially purified renin.

The various batches of renin were compared to a standard renin preparation, using the blood pressure increase of nephrectomized male rats (180–210 g) as index (14). The renin standard (freeze dried) was kept at —16°. One hundred μg of this renin standard increased the blood pressure of nephrectomized rats by approximately 50 mm Hg. This amount of the renin standard was defined as one Standard Laboratory Unit (SLU). In the 2 + 2 point assay doses of 0.1 and 1 SLU were employed. The potency of the renin preparations used in these studies varied from 12 to 24 SLU/mg.

Renin-induced hypertension was produced by subcutaneous administration at each 12-hr interval, of 200 SLU of purified rat renin dissolved in 7% gelatin at 37° (0.1 ml/rat). The gelatin solution contained 1.25 mg of streptomycin and 1250 IU of penicillin/ml.

Aldosterone (6 μg/rat/day), corticosterone (60 μg/rat/day) and dexamethasone phosphate (3 μg/rat/day) were injected twice daily by the subcutaneous route.

Results are expressed as mean ± standard error of the mean (SE). Statistical analysis of the data was performed using Wilcoxon's two sample test (16).

Results. Development of renal hypertension in intact rats and in adrenalectomized rats is shown in Fig. 1. It is clear that renal hypertension in adrenalectomized rats develops in a similar way as in intact rats.

The contradictory reports on the role of the adrenal gland in renal hypertension (see above) might be related to the duration of hypertension. Therefore, the effect of adrenalectomy was also studied in rats with renal hypertension of 12- and 50-days duration (Fig. 2). A slight decrease of blood pressure occurred in both hypertensive groups on the second day following adrenalectomy, but throughout the period studied, hypertension persisted as in the intact rats.

The effect of renin administered to intact rats is shown in Fig. 3. A rise in blood pressure occurred 4–5 days following the first injection of renin. It may be noted that the rise in blood pressure during the development of renal hypertension occurred 1–4 days following the application of the renal artery clip (Fig. 1).

When the same amount of renin was administered to adrenalectomized rats, hypertension failed to develop (Fig. 3). Adminis-

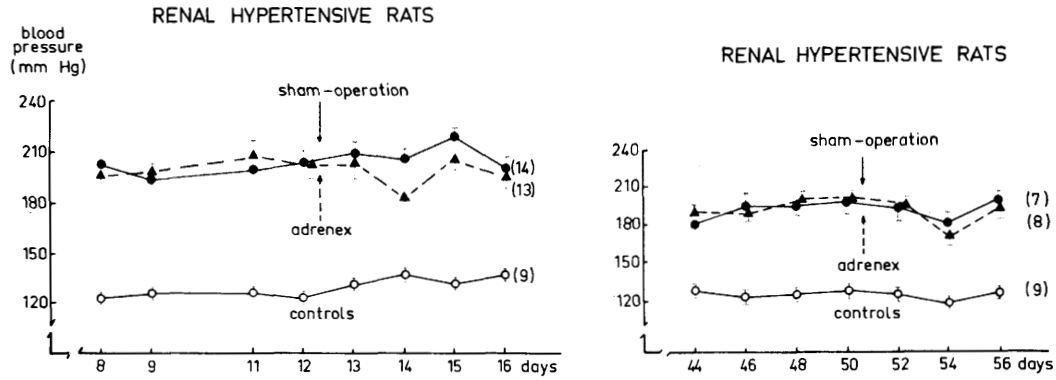

FIG. 2. Effect of adrenalectomy on renal hypertension of 12- and 50-days duration: (filled symbols), rats with renal arterial constriction; (○,●,▲) mean values; bars indicate standard error of the mean; number of rats given in parentheses.

tration of aldosterone or of corticosterone again permitted the renin-induced hypertension to develop in adrenalectomized rats (Fig. 4). To eliminate mineralocorticoid effects, the effect of dexamethasone was also studied (Fig. 6). It is obvious that renin-induced hypertension was established in adrenalectomized rats receiving dexamethasone.

These experiments seem to indicate that renin-induced hypertension differs from renal hypertension by its dependency on a functional adrenal cortex. It occurred to us that such a difference might only be quantitative. Plasma renin activity of adrenalectomized rats with a renal hypertension of 6-days duration was almost twice as high ($p<0.05$) as in intact renal hypertensive rats (Fig. 5). Such high renin levels might not have been reached in the renin-treated adrenalectomized rats. Therefore, the effect of the administration of a gradually increased dose of renin was studied in adrenalectomized rats maintained on 0.9% saline without corticosteroid treatment (Fig. 6). On the first day the previous renin dose (400 SLU/day) was administered; from the second day on twice this dose was given and from the sixth day on the dose was tripled. It is obvious that this course of treatment with higher amounts of renin induced hypertension in adrenalectomized rats, showing that the adrenal cortex and adrenocortical steroids are not essential for the development of renin-induced hypertension.

Discussion. The present studies show a qualitative difference between renin-induced and renal hypertension in adrenalectomized rats maintained on 0.9% saline. In these animals renal hypertension (*i.e.*, following renal artery clip) developed as in intact rats, but an amount of injected renin sufficient to induce hypertension in intact rats failed to

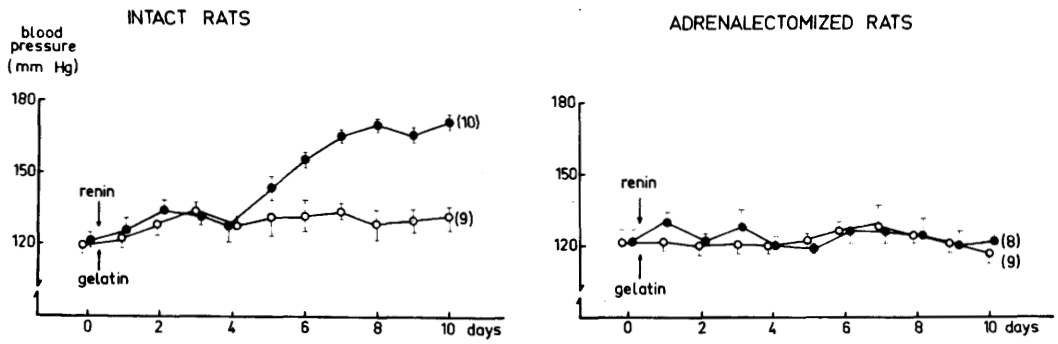

FIG. 3. Renin-induced hypertension in intact and in adrenalectomized rats: (●), rats which received renin; (○,●), mean values; bars indicate standard error of the mean; number of rats given in parentheses.

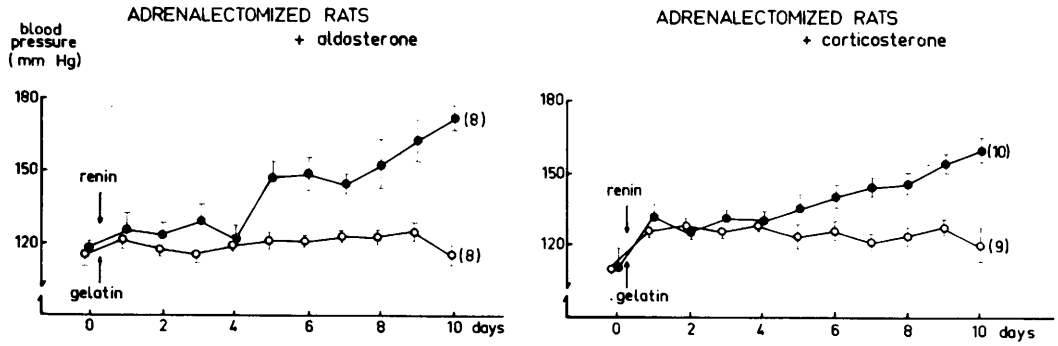

FIG. 4. Renin-induced hypertension in adrenalectomized rats treated with aldosterone or corticosterone: (●), rats which received renin; (○,●), mean values; bars indicate standard error of the mean; number of rats given in parentheses.

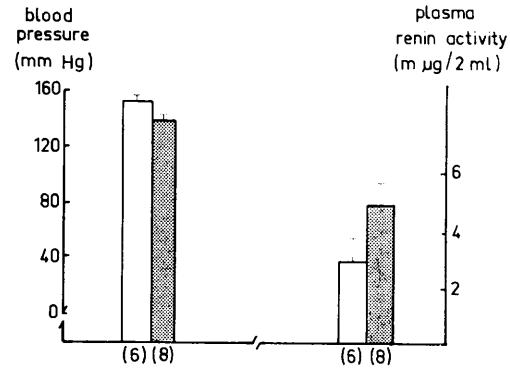

FIG. 5. Effect of renal arterial constriction in intact rats and in adrenalectomized rats on blood pressure and on plasma renin activity: (open columns), intact rats; (stippled columns), adrenalectomized rats; bars indicate standard error of the mean; number of rats given in parentheses.

elevate blood pressure. However, when greater amounts of renin were administered to adrenalectomized rats, hypertension did develop in a similar way as in intact rats. Thus the usual amount of renin administered to adrenalectomized rats may not have increased plasma renin activity to as high a level as that actually achieved in renal hypertensive adrenalectomized rats. It was concluded, therefore, that both for renal hypertension and for renin-induced hypertension the adrenal cortex or adrenocortical steroids are not prerequisites to the development and maintenance of hypertension in the rat. In this respect, the present study reveals no essential differences between renal hypertension and renin-induced hypertension.

The permissive action of corticosteroids on the hypertensive effect of the usual doses of renin used in adrenalectomized rats was also exhibited by dexamethasone in a dose devoid of mineralocorticoid activity (17). Thus, this permissive action seems to be related to glu-

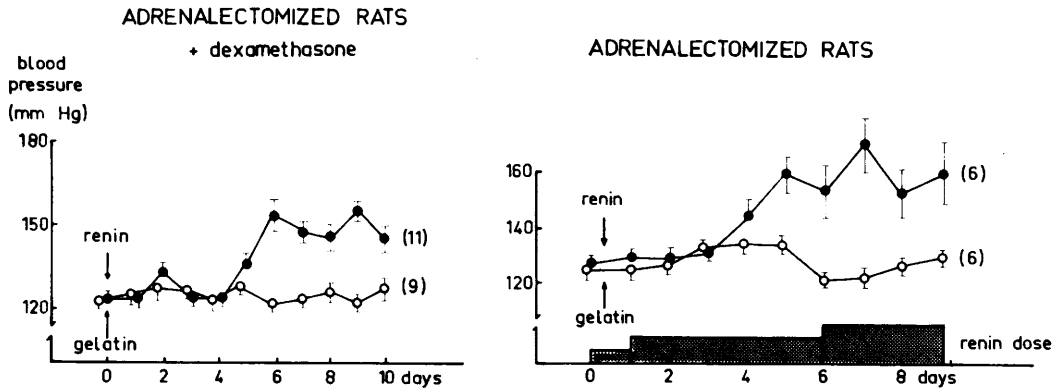

FIG. 6. Effect of renin administration to adrenalectomized rats treated with dexamethasone, and effect of higher doses of renin in adrenalectomized rats without corticosteroid treatment: (●), rats which received renin; (○,●), mean values; bars indicate standard error of the mean; number of rats given in parentheses.

cocorticoid rather than to mineralocorticoid activity. This is substantiated by the work of Masson *et al.* (11), who reported that cortisol restored renin-induced hypertension in adrenalectomized rats, while deoxycorticosterone failed.

The failure of an amount of renin, which is effective in intact rats, to induce hypertension in adrenalectomized rats may depend on several other factors, including the absorption and subcutaneous inactivation of renin. It may also be related to an accelerated rate of metabolic clearance of renin, or to an effect on the biochemical pathway involved in the generation of angiotensin II. However, the possible role of a decreased cardiovascular reactivity following adrenalectomy is particularly note-worthy and is supported by actual data (18–21) as well as by observations that corticosteroids can restore this decreased cardiovascular reactivity (18, 21).

A diminished plasma concentration of renin substrate following adrenalectomy has also been suggested (11) as a cause of the failure of usual amounts of renin to induce hypertension. Although such a mechanism may have played a role in our experiments, it is not a limiting factor as shown in the experiment in which higher doses of renin induced hypertension, not different from that seen in intact rats. Moreover, Doca failed to restore renin-induced hypertension in adrenalectomized rats in studies (11) in which plasma renin substrate concentration was not found to be significantly depressed when compared to intact controls.

Summary. Development of renal hypertension and of renin-induced hypertension was studied in intact and in adrenalectomized rats. Experimental renal hypertension developed similarly in intact rats and in adrenalectomized rats maintained on 0.9% saline. Subcutaneous administration of purified rat renin for 9–10 days induced hypertension in intact rats. Administration of the same amount of renin to adrenalectomized rats maintained on 0.9% saline, however, failed to induce hypertension. Renin-induced hypertension was restored in adrenalectomized rats by the administration of aldosterone, corticosterone, or dexamethasone. These corticosteroids by themselves had no effect on blood pressure of adrenalectomized rats. If larger amounts of renin were administered to adrenalectomized rats, hypertension developed to a similar degree as that observed in intact rats. It is concluded that for the development of both renal hypertension and renin-induced hypertension the adrenal cortex is not essential.

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