PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 126–131 (1974)

Similar Response of Spontaneously Hypertensive and Normotensive Rats to Adrenal Enucleation[1] (38056)

SIMON KOLETSKY, DORITH I. PUTERMAN, AND RICHARD J. KOLETSKY

Department of Pathology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

Although the high blood pressure of spontaneously hypertensive rats (SHR) has a genetic basis (1) its precise etiology is not established. Aoki (2) reported that the adrenal might play a role since removal of these glands in the prehypertensive stage prevented the subsequent development of hypertension, while removal during the course of hypertension caused a lowering of the blood pressure to normotensive levels. On the other hand Nolla-Panades and Smirk (3) questioned the role of the adrenals on the ground that when their genetically hypertensive rats were subjected to adrenalectomy, the animals continued to have high blood pressure when 1% saline was used for drinking instead of tap water.

In the present study participation of the adrenals in the etiology of the high blood pressure in spontaneously hypertensive rats was explored by the technique of adrenal enucleation. The latter removes the medulla and the reticularis and fasciculata zones of cortex leaving in situ the capsule with attached zona glomerulosa. Enucleation is promptly followed by an interval of marked adrenal insufficiency which lasts about one week. Then adrenal cortical function is restored over the next few months (4) as the zona glomerulosa cells proliferate and replace the fascicular and reticularis zones. There is no regeneration of the medulla.

The purpose of the study was to compare the response to adrenal enucleation of SHR and of normal (non-SH) Wistar rats, especially in regard to blood pressure and also to certain other parameters of structure and function of the regenerated adrenal gland. Since the SHR were derived from the Wistar strain by selective inbreeding, their reaction to successive intervals of adrenal hypofunction and restored function might uncover differences in the behavior or appearance of regenerating adrenal cortical cells which resulted from inbreeding and perhaps were etiologically related to the high blood pressure.

Methods. Young adult white male rats 2–3 months of age were used. These comprised the following groups: 10 SHR in the prehypertensive or insipient phase of hypertension which were subjected to right adrenalectomy and enucleation of the left adrenal in a one stage operation; 15 normal rats of the Wistar strain[2] which had the same procedure; and 10 SHR which had a right adrenalectomy and sham enucleation of the left adrenal. All animals were maintained postoperatively on standard diet (Purina Chow) and tap water and remained in good condition under this regimen. No substitution therapy was employed for adrenal insufficiency.

The technique for adrenal enucleation was similar to that of Evans (5) and of Ingle and Higgins (6). Under nembutal anesthesia the adrenal was exposed by a lumbar incision and freed of surrounding fat and the capsule was incised on the side opposite the entry of the blood vessels. By applying

[1] Supported by Grant HL-13990 from National Institutes of Health, U. S. Public Health Service.

[2] Obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass.

gentle pressure to the sides of the gland with a fine forceps, the body of the gland consisting of the entire medulla with attached zona reticularis and zona fasciculata was expressed. Only the capsule with adherent zona glomerulosa cells remained in situ. The same procedure was used for sham enucleation except that no pressure was applied to the adrenal and no tissue was removed.

In order to determine the effect of adrenal enucleation on the blood pressure of SHR and of normal rats, the mean systolic blood pressure of each animal was obtained just before operation and again at 1 week and at 1, 2 and 3 mo after enucleation. The pressures were measured directly by inserting a #50 polyethylene catheter into a femoral or brachial artery and attaching the catheter to a Hg manometer. Using the method of Zenker and Bernstein (7) plasma corticosterone levels were measured in all rats in order to establish the status of adrenal function following enucleation of the gland. Levels were obtained on arterial blood withdrawn just prior to operation and again at one week after enucleation during adrenal insufficiency and at 1 and 3 mo after enucleation during adrenal regeneration. The samples of blood were withdrawn during the afternoon and under nembutal anesthesia.

All animals were sacrificed 3 mo after adrenalectomy and the regenerated left adrenal was removed, dissected free of attached adipose tissue and weighed. Sections of the gland were then prepared for microscopic study with the hematoxylin and eosin stain.

Results. Figure 1 shows the mean blood pressure for the 3 groups of rats before and after adrenal enucleation. At 1 week after operation, i.e., during adrenal insufficiency, both the SHR and the normal rats showed a drop in pressure while there was no drop in the sham enucleated rats which had an intact adrenal gland. Then in the course of adrenal regeneration over the next few months, the blood pressure of the enucleated SHR rebounded to levels that were substantially higher than the preoperative ones. These rats which prior to enucleation were either prehypertensive or had borderline hypertension developed clearcut hypertension, reaching levels of 180–220 mm Hg 3 mo after operation. This contrasted with the moderate elevation in blood pressure which occurred spontaneously over the same period of time in the sham enucleated SHR.

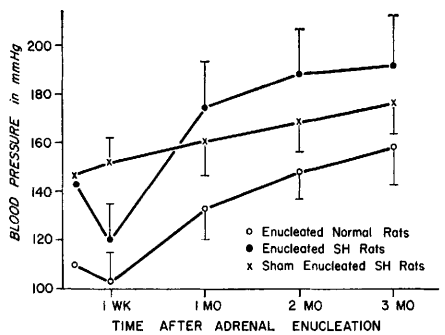

FIG. 1. Blood pressure in rats with adrenal enucleation.

In the course of adrenal regeneration the enucleated normal rats also showed a considerable increase in blood pressure over the preoperative levels. Three of the 15 animals (20%) comprising this group had high blood pressure (150 mm Hg or more) 1 mo after enucleation and at 3 mo 8 or 53% were hypertensive with levels of 156–180 mm Hg.

The curves for the enucleated SHR and for the normal rats are quite similar (Fig. 1). During the stage of adrenal insufficiency 1 week after enucleation the hypertensive animals showed a greater fall in pressure than the normal rats probably because they started at higher levels of pressure before operation. Also the subsequent rebound in blood pressure during adrenal regeneration was greater for the enucleated SHR than for the enucleated normal rats. The sharpest increase in pressure for both groups of animals occurred between 1 week and 1 mo after enucleation and coincided with the interval of most rapid adrenal regeneration. The blood pressure continued to rise, although at a slower rate, between 1 and 3 mo following enucleation during which time regeneration of the adrenal cortex was completed.

Table I shows that the plasma corticosterone levels of the SHR and the normal rats were similar both before and after adrenal

TABLE I. Plasma Corticosterone in Rats with Adrenal Enucleation.

Rat	Before enucleation	After enucleation		
		1 wk	1 mo	3 mo
Enucleated normal	42 ± 4.6[a]	15 ± 3.8	30 ± 4.8	38 ± 4.3
Enucleated SHR	40 ± 12.3	16 ± 3.1	28 ± 4.7	42 ± 9.6
sham Enucleated SHR	35 ± 9.0	35 ± 9.2	36 ± 7.5	40 ± 7.2

[a] Mean ± S.E. in μg/100 ml. N = 8–10 at each time interval.

enucleation. At 1 week after operation there was a considerable reduction in the level of this steroid and then the values rose progressively over the next few months as the adrenal cortex was reconstituted by cellular regeneration. By 3 mo after enucleation the plasma corticosterone of both the enucleated SHR and of the normal rats had returned to the vicinity of the preoperative values in practically all instances. At this time also the peripheral plasma corticosterone levels of the 2 enucleated groups of animals were not significantly different from those of the sham enucleated SHR.

Table II gives the weights of the regenerated left adrenal gland of the SHR and normal rats and also the weights of the intact left adrenal of the sham enucleated SHR. The weights for the 2 enucleated groups of animals indicated a comparable degree of adrenal regeneration. The gross appearance of the glands was similar. Light microscopy showed that the three layers of reconstituted adrenal cortex were similar in width and also in cytological appearance. The zona glomerulosa in both types of rat was relatively narrow. The weights of the regenerated glands in both the SHR and normal rats were almost uniformly lower than those of the intact adrenals from sham enucleated SHR. Perhaps this was due to absence of medulla in the regenerated glands and also to some degree of compensatory hypertrophy of the remaining adrenal in the sham enucleated SHR.

TABLE II. Weights of Regenerated Adrenals Three Months After Enucleation

Rat	Weight[a]
enucleated normal	5.9 ± 0.8
enucleated SHR	6.1 ± 1.2
sham enucleated SHR	7.4 ± 1.0

[a] Mean ± S.E. in mg/100 g body weight. N = 10 for each group of rats.

Discussion. Steroid production by regenerating adrenal glands has been studied both *in vivo* and *in vitro* especially in rats with adrenal regeneration hypertension. The latter has usually been performed in young normal female rats and requires uninephrectomy and a salt load in addition to adrenal enucleation. Plasma corticosterone levels in the peripheral or adrenal venous blood of rats with adrenal enucleation were reported by a number of investigators to have returned to the vicinity of normal by the time regeneration was complete (8–14). At no time during adrenal regeneration have sufficiently elevated peripheral levels of plasma corticosterone been found which could cause hypertension.

There is general agreement that the production of aldosterone by the regenerating enucleated adrenal remains at low levels throughout the entire period of regeneration (11, 13–15) and hence that this steroid is not responsible for elevation of the blood pressure. For example Brogi and Pellegrino (11) found that aldosterone levels in

adrenal venous blood at 1 and 2 mo after enucleation were much below normal control values. Several authors reported that aldosterone secretion by incubated regenerated adrenal glands was considerably reduced or impaired in comparison with normal adrenals (10, 16, 17). Chemical analysis of steroids released during incubation by regenerating adrenals removed at 3 weeks or 1 mo after enucleation showed diminished secretion of corticosterone, aldosterone and 6-β-OH-DOC as compared to normal adrenals and also no significant imbalance in the production of these steroids (10).

Several studies provided evidence that increased secretion of DOC by the regenerating adrenal plays a role in the origin of adrenal regeneration hypertension. From *in vitro* studies of homogenized incubated adrenal glands Brownie and Skelton (18) and later Skelton and Brownie (8) reported that regenerating adrenals had reduced 11-β-hydroxalase activity which retarded conversion of precursor to corticosterone and resulted in excess production of DOC. It was suggested this might be the cause of adrenal regeneration hypertension. While not confirming increased production of DOC *in vitro,* Rapp (13) reported that the peripheral plasma levels of DOC in rats 4 weeks after adrenal enucleation were substantially higher than in normal rats and that the increase was probably sufficient to account for the high blood pressure in these animals. Brown and coworkers (19) found that immediately after adrenal enucleation the levels of peripheral plasma DOC were nearly unmeasurable but then in the course of adrenal regeneration rose rapidly to a peak at 3 weeks and were markedly elevated between 2 and 7 weeks after enucleation, i.e., while the blood pressure was rising to hypertensive levels. Grekin *et al.* (14) on the other hand reported that the levels of DOC in adrenal venous plasma of enucleated rats were not elevated during the course of adrenal regeneration. However these authors attached some significance to the fact that following enucleation DOC was the first steroid to return toward normal and was nearly back to normal by 3 weeks at a time when the levels of other steroids were still very low. This finding was thought to lend support to the contention that DOC played an important role in adrenal regeneration hypertension.

Increased *in vitro* production of 18-OH-DOC by incubated regenerating rat adrenal was reported by several authors for glands studied from 1–2 mo after enucleation (20–22). However Rapp (23) found that the peripheral plasma levels of this steroid in rats 4 weeks after adrenal enucleation were not significantly different from those of control rats. Grekin *et al.* (14) stated that the amounts of 18-OH-DOC in adrenal vein rat plasma were reduced at 3 weeks after adrenal enucleation, i.e., at a time when the blood pressure was rising, and elevated at 6 weeks and hence that the steroid might be a contributing factor to the increasing hypertension at the latter time.

Some authors proposed a different basis for the high blood pressure which develops in rats with adrenal regeneration. Grollman (24) suggested that the electrolyte disturbances which occurred in enucleated rats during adrenal insufficiency together with the factors of nephrectomy and salt load were responsible for the high blood pressure. Masson *et al.* (10) ascribed the hypertension to a sensitizing of the vascular tree during the period of adrenal hypofunction which later enhanced its response to the normal hormones produced by the regenerating adrenal. If theses of this nature are correct, the fall in blood pressure observed in our animals shortly after enucleation and during the interval of adrenal insufficiency may have been due to such factors as depletion of body sodium and water, electrolyte shifts in the vascular tree, hypovolemia, and reduced cardiac output. These changes were presumably corrected as a result of adrenal regeneration and the correction was accompanied by a rebound of the blood pressure. The latter carried to levels which were substantially higher than the preoperative levels and such overshoot would be consistent with the presence of a hyperreactive arteriolar bed.

A majority of the normal rats used as controls in this study developed high blood pressure following adrenal enucleation. This

high incidence was unexpected since the animals were not subjected to reduction in renal mass by uninephrectomy and to salt load which, as stated, form part of the standard procedure for inducing adrenal regeneration hypertension. Perhaps the relatively high content of NaCl in the Purina chow diet was a contributing factor.

Both the SHR and the normal rats in the present study reacted to adrenal enucleation in like manner. The curves of blood pressure response of the 2 types of animal were similar except that the initial fall and subsequent rebound of pressure were greater in the hypertensive rats. This may have been due to the higher starting levels of pressure in the SHR prior to enucleation and also to the fact that during the rebound the underlying cause of the high blood pressure in SHR was operative as an additional factor. The hypertensive levels reached by the enucleated SHR were well in excess of those for the sham enucleated SHR and this illustrates the accelerating effect of adrenal regeneration on the blood pressure. However the increments in pressure of the enucleated SHR which theoretically might represent the additive effects of enucleation and the spontaneous rise in pressure expected of SHR were less than the combined increments of the enucleated normal rats and the sham enucleated SHR.

Both the hypertensive and normotensive animals showed a comparable reduction in plasma corticosterone in the interval of adrenal insufficiency after enucleation of the gland and then a similar rise in the level of this steroid during regeneration of the adrenal cortex. When restoration of the adrenal cortex was complete 3 mo after enucleation, the weights of the regenerated glands of SHR and normotensive rats were of comparable magnitude and with light microscopy no significant difference was detected in the structure of the glomerulosa, fasciculata and reticularis zones of adrenal cortex.

Thus in respect to several parameters, both structural and functional, the regenerating adrenal cortex of SHR behaved like that of normal rats. Regeneration of the adrenal cortical cells of SHR did not disclose any inherent or distinctive property of these cells in comparison with the corresponding cells of their progenitors. In both enucleated SHR and enucleated normal rats restoration af adrenal function through cortical regeneration was accompanied by the development of hypertension. While our study indicates a similar origin for the high blood pressure in both types of animal, it does not define the precise etiology. The regenerating adrenal played either a permissive role by providing the milieu in which hypertension due to other factors could develop, or was causative as a result of abnormal secretion or hypersecretion of steroids.

Summary. Spontaneously hypertensive rats (SHR) and normal Wistar rats were subjected to right adrenalectomy and enucleation of the left adrenal. Both types of animal responded to this procedure in similar manner. The blood pressure fell during the initial interval of adrenal insufficiency and then rebounded during adrenal regeneration to substantially higher levels than the preoperative. Slightly more than half the normal rats were rendered hypertensive by adrenal enucleation. Plasma corticosterone declined shortly after enucleation and then rose progressively to normal levels in the course of adrenal regeneration. Three months after enucleation the adrenals of both groups of animals were similar in weight and also in gross and microscopic appearance. In the parameters of function and structure studied, the regenerating adrenal cortical cells of SHR showed no distinct difference from the corresponding cells of the progenitor strain. In both the SHR and the normal Wistar rats the regenerating adrenal either provided the milieu which permitted hypertension to develop or caused the high blood pressure through hypersecretion or abnormal secretion of steroids.

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Received Dec. 3, 1973. P.S.E.B.M., 1974, Vol. 146.