Mineralocorticoid Hypertensive Cardiovascular Disease Induced in Hypophysectomized Rats

BY C. E. HALL, PH.D., O. HALL, M.S.C., AND E. G. RENNELS, PH.D.

EXPERIMENTAL hypertensive cardiovascular disease is easily induced in the rat by desoxycorticosterone acetate (DCA) especially, although by no means exclusively, in animals sensitized by augmented sodium intake and removal of a kidney.\textsuperscript{1,2} Other mineralocorticoid steroids, differing quantitatively in potency, have the same effect.\textsuperscript{3,4} Depending upon the circumstances of the experiment, either benign or malignant hypertension may develop in the rat; such responses command much interest because of their possible relationship to certain forms of human hypertensive disease.

The role of various endocrine glands in the etiology and pathogenesis of mineralocorticoid-induced hypertensive disease has been studied in some detail. Pituitary dependency has been suggested by reports that hypophysectomy prior to DCA treatment prevents hypertension,\textsuperscript{5-7} whereas if performed on animals already hypertensive will restore the blood pressure to normal, regardless of whether steroid treatment is stopped,\textsuperscript{8,9} or continued.\textsuperscript{10,11}

This reported indispensability of the pituitary gland has led to the view that desoxycorticosterone requires for its hypertensive effect some specific hypophysial hormone of either known or unknown nature. Recently, however, it has been possible to demonstrate that DCA produces hypertensive cardiovascular disease in the sensitized rat in which the hypophysis has been removed from the sella turcica and transplanted to the renal capsule.\textsuperscript{12} Such autografts are known to secrete significant amounts of luteotrophin (LTH), but either greatly reduced or undetectable amounts of other anterior hypophysial principles.\textsuperscript{13} Hence, although they readily developed hypertension, DCA-treated hypophysectomized autografted animals failed to grow and evidenced extreme atrophy of the thyroids, adrenals and gonads. Although this demonstrated that DCA was effective in the absence of significant quantities of known pituitary principles (other than LTH), it still remained possible that the response required either trace amounts of these, some unknown principle secreted by the autografts, or perhaps LTH itself. Therefore, a re-examination of the alleged antihypertensive effect of hypophysectomy in animals treated with DCA was undertaken.

Methods

The experimental series consisted of 57 female rats of the Holtzman strain, weighing 100 to 120 Gm., subdivided into 6 groups. Animals of groups 1 (8 rats) and 2 (14 rats) were hypophysectomized (hypox); groups 3 (8 rats) and 4 (10 rats) were also hypox, but in these the pituitary gland, after removal, was transplanted beneath the capsule of the left kidney; groups 5 (9 rats) and 6 (8 rats) consisted of intact animals. All were later subjected to right unilateral nephrectomy, a pellet of crystalline DCA,* weighing 70 to 75 mg., was implanted subcutaneously into animals of groups 2, 4, and 6, and all animals, individually housed, thereafter received a drinking solution containing 0.85 per cent NaCl and 5 per cent sucrose, and Purina Laboratory Chow \textit{ad lib}.

Hypophysectomized animals which either grew appreciably or acquired the coarse fur indicative of pituitary activity were eliminated from the experiment. Blood pressures were taken periodically on unanesthetized animals using a tail plethysmograph. Body weights were taken at weekly intervals. Fluid intake was measured over a 24-hour period at 4 intervals during the experiment as an estimate of polydipsia.

\footnotesize{\textsuperscript{*}Cortate pellets were generously supplied by the Schering Corporation, Bloomfield, N. J.}

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On the eighty-ninth day of the experiment, the animals were killed with ether. The viscera were examined for lesions, the sella turcica of the hypophysectomized animals for pituitary remnants and the renal capsule of autograft-bearing remnants for the transplant. Various tissues were fixed in 10 per cent neutral formalin for histologic preparation, and after fixation, those to be weighed were removed, dissected out, blotted carefully and weighed on a delicate tension balance. Vascular lesions in the hearts and kidneys, and arteritis of the mesenteric and pancreatic vessels, determined histologically, were arbitrarily graded on a scale of severity from 0 to 3 as described elsewhere, and the group score divided by the number of component animals to arrive at an estimate of comparative severity.

Results

Growth

Hypophysectomized animals failed to grow postoperatively. Those with pituitary autotransplants grew slightly, suggesting a limited ability of these to secrete growth hormone. This may have been pre-formed hormone, and is not construed as necessarily indicative of continued hormone synthesis. Growth was unaffected by simultaneous DCA treatment. Animals with intact pituitaries grew normally, again uninfluenced by DCA until induced illness produced cachexia. The growth curves are given in figure 1.

Blood Pressure

DCA treatment of intact animals rapidly induced a severe and fatal hypertension, which, in either hypophysectomized or autografted animals, was slower in onset and progress. Ultimately all of the autotransplanted and all except one of the hypophysectomized animals developed hypertensive disease. Pressures above 150 mm. Hg eventually developed in 4 of the intact controls, presumably reflecting a salt hypertension. This did not, however, occur in either the hypophysectomized or autotransplanted groups consuming NaCl solution but not receiving hormone. The blood pressure findings are given in table 1.

Fluid Intake

Measurements over a 24-hour period on 4 occasions during the experiment revealed that animals in each of the DCA-treated groups consumed more fluid than their untreated counterparts and, in both steroid-treated and control groups, consumption by rats bearing autotransplants was intermediate between that of hypophysectomized and intact animals, as were the body weights. The data are summarized in figure 2.

Health

Consonant with earlier findings, intact, sensitized, DCA-treated animals proved unable to withstand treatment; all were dead by the end of the tenth week, terminally developing severe cachexia. At autopsy, renal and cardiac lesions, usually of great severity, were noted. Hypophysectomized or autotransplanted DCA-treated rats, even those with pressures greater than 200 mm. Hg, were not, in general, as adversely affected thereby. However, a few deaths occurred among them toward the end of the experiment, and in these, at autopsy, periarteritis was grossly visible.

Organ Weights

Severe and presumably complete atrophy of the adrenals, thyroids and ovaries characterized all hypophysectomized animals regardless of the pres-
Figure 2
Effect of desoxycorticosterone treatment on the consumption of saline-sucrose drinking fluid by intact, hypophysectomized and hypophysectomized autotransplant-bearing rats. As indicated by the bracketed numeral, only 1 of the DCA-treated intact animals survived until the sixty-ninth day.

Figure 2

Effect of desoxycorticosterone treatment on the consumption of saline-sucrose drinking fluid by intact, hypophysectomized and hypophysectomized autotransplant-bearing rats. As indicated by the bracketed numeral, only 1 of the DCA-treated intact animals survived until the sixty-ninth day.

ence or absence of transplants. In the case of the adrenals and thyroids, DCA treatment did not further modify the atrophy, but in hypox animals, with or without transplants, the ovaries of DCA-treated animals were about twice as large, in proportion to body weight, as in untreated controls. At autopsy, the ovaries of DCA-treated females were red and hyperemic, contrasting markedly with the pale color in those not receiving steroid. Hypertensive cardiac enlargement and renal hypertrophy were clearly evident among surviving DCA-treated animals. Hearts and kidneys of these animals were proportionately larger than in untreated animals (in each case P<.001). These findings are in conformity with the observed hypertension (table 1).

Vascular Pathology
All animals treated with DCA, with the single exception of 1 hypox animal whose blood pressure reached only a "pre-hypertensive" level of 145 mm.Hg, were found to have vascular lesions in the heart, kidney, pancreas, mesentery or spleen, and often all were involved.

Intact animals, although none survived the full period of treatment, showed the most severe renal and cardiac lesions. Nephroscle-rosis was typically so severe that normal nephrons could not be found in the microscopic sections. Arteriolar necrosis, necrotizing glomerulitis, tubular casts and tubular necrosis were everywhere common. In the hearts, myocarditis, fibrosis, vascular and myocardial necrosis, and granuloma formation were characteristic. Periarteritis of the spleen, mesentery and pancreas was moderately severe when present, although not evident in those animals which died early in the experiment.

Although in many instances the blood pressure had exceeded 200 mm.Hg for 6 weeks prior to autopsy, autotransplanted animals showed less renal injury. Much healthy tissue remained, and casts were far less common. Although glomerular hyalinization was present, fewer glomeruli were so affected, and the arterioles were rarely involved. Cardiac lesions were of about the same frequency, although less severe than in animals with an intact hypophysis. Periarteritis of the visceral vessels was, however, much more prominent and extensive than in the latter.

Hypophysectomized animals were even less affected by renal and cardiac lesions. The kidneys of all hypertensive hypox animals bore lesions, arteriolar necrosis was not observed, but glomerular hyalinization was evident. Curiously from 1 to 3 or 4 completely hyalinized glomeruli might be present in a given section—always smaller than in similarly affected intact animals—with the remainder showing little or no alteration. Such glomerular lesions showed none of the capillary thickening and swelling so characteristic of hypertensive intact animals, and were, although individually severe, infrequent. Tubular segments were rarely affected except by dilatation and tubular casts were exceedingly scarce. The over-all impression gained of the most severely involved kidneys was that about 90 to 95 per
Table 1

Effect of Pituitary Ablation or Autotransplantation to the Renal Capsule on Responsiveness to DCA Treatment.

<table>
<thead>
<tr>
<th>Data</th>
<th>Pituitary removed Control</th>
<th>Pituitary autotransplanted Control</th>
<th>Pituitary intact Control</th>
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<tr>
<td></td>
<td>DCA</td>
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<td>DCA</td>
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<td>No. of rats</td>
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</tr>
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<td>8</td>
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<tr>
<td>Final</td>
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<tr>
<td>27th</td>
<td>123±1*</td>
<td>146±7</td>
<td>122±2</td>
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<td></td>
<td>178±8</td>
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<tr>
<td>Heart</td>
<td>334±7</td>
<td>495±34</td>
<td>305±9</td>
</tr>
<tr>
<td></td>
<td>519±14</td>
<td>362±14</td>
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<td>Kidney</td>
<td>653±17</td>
<td>824±26</td>
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<td></td>
<td>812±18</td>
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<tr>
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<td>Av. severity</td>
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*Mean±S.E. of mean.
†Single survivor.
‡Periarteritis of mesentery, spleen and pancreas.

Discussion

In consonance with our earlier findings, hypertensive cardiovascular disease resulting from DCA treatment occurred in all animals with pituitary glands, regardless of whether these were left in situ or relocated beneath the renal capsule. In contradistinction to what others have found, however, the same was true of hypox animals, negating both the view that hypophysectomy prevents DCA-induced hypertension and the supposition that some specific pituitary secretion necessarily mediates the hypertensive response to the steroid.

However, the rapid development of hypertension and the extensive renal and cardiac lesions in intact animals contrasted with the slower progress of the disease and the paucity of lesions in those whose pituitary gland had been either transplanted or removed. This leaves little doubt that pituitary function is capable of altering profoundly both the ease with which the disease can be induced and the severity and distribution of the consequent vascular lesions. In respect to growth, fluid intake, and the cardiorenal lesions, DCA-treated animals with pituitary transplants occupied an intermediate position between similarly treated intact and hypophysectomized animals.

In view of the numerous reports indicating that DCA fails to produce hypertension in hypox animals, some comment on its success in the present instance seems warranted. Our
Figures 3-6. (See legends on opposite page)

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experience has been that high doses of DCA are damaging to hypox rats on high NaCl intake, although the implantation of small 70 to 75 mg. pellets is well tolerated. Experiments in which DCA lacked effect have, for the most part, been of short duration. This may well account for the absence of nephrosclerosis, which develops slowly and is less extensive in DCA-treated hypophysectomized rats, but hardly explains the failure to produce hypertension, easily demonstrable after 3 to 4 weeks of treatment. In many of the reported experiments, however, blood pressures were not measured. Finally, our hypox animals have uniformly received a drinking solution containing (in addition to NaCl) sucrose, which improves their general condition. The carbohydrate does not appear to have been used by other investigators, and it may well be that the ability of DCA to produce hypertension in hypox animals requires the correction of some defect in metabolism which dietary sucrose supplementation achieves. Further, the present experiment utilized female rats, whereas the others cited employed males. Thus, the possibility of a sex influence—even in hypophysectomized rats—although deemed unlikely, cannot be entirely discounted.

Renal and cardiac lesions were less evident in DCA-treated animals whose hypophysis had either been ablated or relocated in the kidney, contrasting markedly with their exuberant development in animals with intact pituitary glands. However, the severity of periarteritis showed the reverse relationship. Intact animals succumbed rapidly—apparently as a result of extensive cardiac, renal and cerebral vascular disease—before arteritis of the mesenteric and pancreatic vessels had time to develop to the advanced degree apparent in similarly treated but longer surviving hypox animals. Hypophysectomy thus appears to protect arterioles, but not the small arteries against the effects of hypertension. The pattern of lesions in the DCA-treated hypox rat is remarkably like that said to typify the similarly treated thyroidectomized animal.16

Surprisingly, a recent review of hypertensive disease17 dismissed mineralocorticoid hypertension as a form of renal hypertension because “renal damage is evident and the hypertension is attributable to this renal injury.” This view fails to take cognizance of the fact that DCA is capable of inducing hypertension which persists after steroid treatment is stopped, often in the absence of renal lesions which can be held culpable for the hypertensive state,8 and that renal lesions identical in

Figure 3
A. Nephrosclerosis in DCA-treated intact animal. Glomeruli show hyalinization, fibrinoid necrosis and shrinkage. Tubular necrosis and distention with colloid casts prominent. H. and E. × 86.
B. Same section at higher magnification. Capillary endothelium is thickened and adherent to wall of Bowman’s capsule. × 172.

Figure 4
A. Kidney section from DCA-treated hypertensive hypox animal. Tissue is essentially normal, although arrows indicate 2 hyalinized glomeruli and 1 or 2 colloid casts are visible. H. and E. × 86.
B. Same section at higher magnification. Damaged glomeruli, indicated by arrows, are not nearly as enlarged as those of intact animal above; and no thickening of capillary endothelium is detectable. × 172.

Figure 5
Fibrinoid necrosis of the wall of a cardiac artery, surrounded by inflammatory cells. From a DCA-treated, hypertensive, hypophysectomized rat. H. and E. × 86.

Figure 6
Periarteritis of mesenteric vessels in hypophysectomized rat, hypertensive from DCA treatment. Intimal and medial necrosis and surrounding inflammatory reaction. H. and E. × 86.
nature and severity to those observed in hypertensive animals may be found in normotensive animals which have been "cured" of a pre-existing hypertension. Furthermore, although DCA treatment caused many of the hypox animals to become as hypertensive as similarly treated intact animals, and for an even longer period, renal and cardiac lesions were rare in the former and exceedingly widespread and severe in the latter. Since hypertension was as marked in hypox animals with few or no renal lesions as in intact animals which had the most widespread nephrosclerosis, the ascription of hypertension to renal lesions would seem to put a shadowy cart before a rather substantial horse. The vascular lesions appears to be caused by the hypertension and hypophysectomy either reduces the sensitivity of renal and cardiac vessels to the deleterious effects of elevated arterial pressure, or diminishes the consequent vascular reaction. The disappearance of papilledema, headache, dyspnea and typical electrocardiographic changes—in the face of unaltered hypertension—which follows total adrenalectomy for malignant hypertension in man, has been explained in precisely this way.

The physiologic derangement underlying mineralocorticoid induced hypertension is in dispute, although, since hypertension fails to occur if sodium is kept from the diet and is aggravated by increasing intake of this cation, some aspect of sodium metabolism and extracellular fluid balance is clearly implicated. The suggestion has been made that increased sodium content of arterial walls may be basic. Whatever the role of sodium ion in the genesis of hypertensive disease, it seems not dependent solely upon the quantity ingested, but probably upon the retention and distribution of that consumed. For although it was true that the various groups of DCA-implanted rats consumed more saline than did their respective controls and became hypertensive, it is equally true that the intact, untreated controls consumed much more saline than did either autotransplant-bearing or hypox DCA-treated rats. Despite the lower saline consumption, all save one of the DCA-treated hypox and autotransplant-bearing rats developed hypertension, whereas only a few of the polydipsic intact untreated controls did so. Clearly, whatever the nature of the basic disturbance, it can and does occur in the absence of the pituitary gland and its hormones.

Summary

Reports in the literature to the contrary, hypertensive cardiovascular disease proved to be readily induced by desoxycorticosterone in hypophysectomized rats sensitized to steroid action by partial nephrectomy and high NaCl intake. In the absence of the pituitary, however, hypertension was somewhat slower in onset and progress. Although hypophysectomized animals were able to develop hypertension as severely as did intact animals, they were not, as judged by their better survival and less evident cardiorenal vascular lesions, as severely injured thereby.

Contrasted with their exuberant development in hypertensive intact animals, renal lesions in hypertensive hypophysectomized animals were rare. Necrotizing arteritis in the heart was seen in about half of the hypox animals, and periarteritis nodosa of pancreatic and mesenteric arteries was more widespread and severe than in similarly treated intact animals which had, however, died earlier.

Steroid treated animals bearing pituitary autotransplants beneath the renal capsule, developed more severe and widespread cardiorenal lesions than did similarly treated hypophysectomized animals, but incidence and severity of periarteritis nodosa in splanchnic arteries was about the same in each.

The ingestion of sodium chloride caused "salt hypertension" in some of the pituitary-bearing controls, but not in either hypophysectomized or autotransplant-bearing controls.

Summario in Interlingua

In despecto do reportos del contrario in le literatura, morbo cardiovascular hypertensive se provava facilmente inducibile per medio do desoxycorticos terona in hypophysectomisate rats que habeva esse sensibilisate al efecto de steroides per nephrectomia partial e un ingestion elevato de NaCl. Tales, in le
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absencia del glandula pituitaria, tanto la declaración como etiam le progreso del hypertension esseva un paucio retardate. Ben que hypophysectomisate animales esseva espanso a disveloppamento le mesme sever grades de hypertension como le animales intacte, a judicar per lor meliorate superviventa e lor minus evident le lesiones vasculo-cardiorenal, illos non esseva injuriate per le hypertension tanto severemente como le animales intacte.

Per contrasto con le exuberante disveloppamento le lesiones renal in hypertensive animales intacte, tal lesiones esseva rar in hypophysectomisate animales hypertensive. Arteritis necrotisante in le corde esseva constatatate in circa un medietate del hypophysectomisate animales. Periarteritis notosce del arterias pancreatica e mesenteric esseva plus extense e plus sever in le hypophysectomisate animales que in le intacte que alteremente habeva recipite le mesme tractamento, sed le animales intacte habeva morte plus proccedere. Animales tractate con steroide le quales portava autotransplanatazione pigmenti infra le capsula renal disveloppava plus sever e plus extense lesiones cardio-renal que le hypophysectomisate animales que habeva alteremente recipite le mesme tractamento, sed le incidente o severitate de periarteritis notosce in arterias splanchnic esseva circa identic in le du grupps.

Le ingestion de chloruro de natrium causava "hypertension de sal" in certes del animales de controlo que habeva essite pertension de sal, in le animales de controlo que habeva essite perle hypertension, nephrosclerosis and cardiac hypertrophy in the rat by desoxycorticosterone acetate overdosage. Am. Heart J. 27: 338, 1944.


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