Prevention of Methylandrostenediol, Methyltestosterone and Testosterone-Induced Hypertension in the Rat by Hypophysectomy (36905)

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It is still controversial whether or not the administration of deoxycorticosterone acetate (DOCA) to hypophysectomized rats induces hypertensive vascular disease. According to several authors (1–4), DOCA treatment fails to produce hypertension in these animals whereas according to others (5) hypertension does occur, although the lesions typical of the disease are less severe than those seen in normal rats. More recently, it was demonstrated that hypertensive vascular disease, identical in its morphological appearance to that produced by DOCA, is induced in the rat by administration of androgens such as methylandrostenediol (MAD) (6), methyltestosterone (MT) (7), and testosterone (8, 9). It was also shown that the hypertensive action of these androgens is indirect and the consequence of an increased production of deoxycorticosterone (DOC) attributable to an impairment of the normal steroid biosynthesis in the adrenal glands of rats receiving these androgens (7–10). Furthermore, high levels of DOC were measured in the plasma (9, 11) in MAD and testosterone-treated animals.

Therefore, in view of the relationship between DOC-induced hypertension and that induced by androgens, we carried out the present investigation to ascertain whether removal of the pituitary could modify the incidence of androgen-induced experimental hypertensive vascular disease.

Materials and Methods. Ninety day old female rats of the Sprague-Dawley CD strain, supplied by Charles River, Inc. were used. All animals were right nephrectomized, given free access to 1% NaCl drinking solution, fed a diet containing 0.5% NaCl with the daily addition of one eighth of a fresh orange and maintained in a room kept at 28°, with 12 hr light and dark cycles. Three days after nephrectomy some of the rats were hypophysectomized. After 1 wk of rest all rats, whether or not hypophysectomized, were divided into the following groups:

Group 1 (controls) consisted of 10 rats receiving a subcutaneous daily injection of 0.2 ml of corn oil for 5 days a week. Groups 2, 3, and 4 consisted of 10 rats each, injected, respectively, with 10 mg of MAD or 20 mg of methyltestosterone or 10 mg of testosterone dissolved in 0.2 ml of corn oil. Group 5 consisted of 16 hypophysectomized rats receiving 0.2 ml of corn oil. Groups 6, 7, and 8 also consisted of 21 hypophysectomized rats each receiving, respectively, 10 mg of MAD, 20 mg of methyltestosterone or 10 mg of testosterone dissolved in 0.2 mg of corn oil. For all of these groups the schedule and administration route was the same as of the controls.

The treatment was carried on for 6 wk and the rats were killed by decapitation at the end of this time period. Body weight and systolic blood pressure were recorded in each animal at the beginning and end of the experiment. Systolic blood pressure was determined in the tail with a Physiograph Four (E & M instrument Co., Houston, TX), using a technique reported previously (7). Plasma concentrations of sodium and potassium were determined with a Beckman B flame photometer. All organs to be studied histologically were placed in a 10% buffered formalin, trimmed after fixation and weighed. Representative blocks were embedded in paraffin, cut at 5 μm and stained by the periodic

[1] Deceased, Oct., 1969.

TABLE I. Effect of Methylandrostenediol, Methyltestosterone and Testosterone on the Body Weight and the Systolic Blood Pressure of Normal and Hypophysectomized Rats.

Treatment	Treatment (wk)	No. of rats	Body wt (g)		Systolic blood pressure (mm Hg)	
			Initial	Final	Initial	Final
Controls	6	10	130 ± 7[a]	247 ± 2	96 ± 2	101 ± 2
Methylandrostenediol	6	10	128 ± 6	236 ± 8	91 ± 3	148 ± 5[b]
Methyltestosterone	6	10	132 ± 2	256 ± 5	95 ± 3	140 ± 7[b]
Testosterone	6	10	133 ± 3	253 ± 8	96 ± 4	138 ± 5[b]
Hypophysectomized	6	16	128 ± 2	121 ± 2	98 ± 2	77 ± 2
Hypophysectomized + methylandrostenediol	6	21	128 ± 2	114 ± 2	90 ± 2	79 ± 2
Hypophysectomized + methyltestosterone	6	21	128 ± 2	114 ± 2	94 ± 2	79 ± 2
Hypophysectomized + testosterone	6	21	128 ± 1	118 ± 2	92 ± 2	83 ± 3

[a] Standard error of the mean (SEM).
[b] $p < .01$.

acid–Schiff procedure. All numerical data were analyzed according to Student's "t" test for small sample size, and throughout the remainder of this report the differences between means having a p value $< .05$ are considered significant and a p value of $< .01$ are considered highly significant.

Results. It is shown in Table I that animals with an intact hypophysis gained in weight during the experiment. The hypophysectomized rats lost weight and such loss was not corrected by the administration of MAD, MT or testosterone. A significant increase in systolic blood pressure was evident in normal rats receiving the androgens and among these animals, those receiving MAD showed the highest increase. On the other hand, in hypophysectomized rats, the systolic blood pressure decreased significantly whether or not the rats were treated with the three androgens (Table I).

An increase in plasma sodium concentrations was evident in all animals hypophysectomized or not, treated with the three steroids. Such an increase, however, was significant only for the intact rats receiving MAD. Plasma concentrations of potassium were slightly increased in intact animals receiving methyltestosterone and testosterone, and slightly decreased in all hypophysectomized rats receiving androgens (Table II).

In Table II are also reported the weights of kidneys, hearts, thymus, adrenals, and ovaries. Hypophysectomy caused a decrease in the weight of all of these organs which was particularly evident in the thymus, adrenals, and ovaries. Androgen treatment produced an increase in the weight of kidneys and hearts in both normal and hypophysectomized rats. This increase, however, was more evident in the intact rats. Steroid administration produced a marked reduction of the thymus weight in all rats. A significant decrease was seen in the weight of adrenals and ovaries in the intact rats receiving MAD, MT or testosterone, while administration of the same hormones in the hypophysectomized animals produced a very modest increase in the weights of both glands which became significant for the rats receiving testosterone.

The kidneys, hearts, and the arterioles of the pancreatic mesentery of the hypertensive rats receiving androgens had gross and microscopic lesions typical of the disease and already extensively described (6–9). On the other hand, the same organs in hypophysectomized rats treated with the three androgens did not present any such gross or histologic changes. The only modification was a slight enlargement of the kidneys which were paler than those of the controls. At microscopic examination, some tubular cloudy swelling

TABLE II. Plasma Sodium and Potassium Concentrations and Weights of Pertinent Organs of Normal and Hypophysectomized Rats Treated with Methylandrostenediol, Methyltestosterone, and Testosterone.

Treatment	Plasma sodium (mEq/liter)	Plasma K (mEq/liter)	Weight (mg)				
			Kidney	Heart	Thymus	Adrenals	Ovaries
Controls	143 ± 1[a]	5.4 ± 0.1	1443 ± 61	840 ± 61	492 ± 56	66.5 ± 3.2	66.3 ± 3.5
Methylandrostenediol	148 ± 2[b]	5.3 ± 0.2	2795 ± 60[c]	1125 ± 29[c]	128 ± 27[c]	51.0 ± 2.0[b]	26.7 ± 2.4
Methyltestosterone	146 ± 1	6.0 ± 0.3	2612 ± 165[c]	1215 ± 59[c]	125 ± 22[c]	49.7 ± 1.8[b]	21.2 ± 1.5
Testosterone	146 ± 2	6.4 ± 0.3[b]	2475 ± 128[c]	1066 ± 33[b]	126 ± 29[c]	50.5 ± 2.5[b]	23.1 ± 2.0
Hypophysectomized	142 ± 1	5.8 ± 0.2	580 ± 19	508 ± 14	135 ± 9	8.7 ± 0.6	9.2 ± 0.7
Hypophysectomized + methylandrostenediol	144 ± 1	4.5 ± 0.5	718 ± 30[b]	540 ± 19	39 ± 11[c]	9.3 ± 1.2	11.3 ± 1.1
Hypophysectomized + methyltestosterone	144 ± 1	4.7 ± 0.6	681 ± 14[b]	511 ± 14	29 ± 5[c]	9.9 ± 0.9	12.0 ± 1.0
Hypophysectomized + testosterone	143 ± 1	4.9 ± 0.5	698 ± 20[b]	552 ± 18	22 ± 3[c]	10.3 ± 0.5[b]	12.4 ± 1.0[b]

[a] Standard error of the mean (SEM).
[b] $p < .05$.
[c] $p < .01$.

and a few tubular hyaline casts were seen in these kidneys. Adrenals of androgen-treated rats bearing the pituitary gland showed vacuolization and formation of PAS-positive colloid droplets in the cells of a zona fasciculata. In the hypophysectomized animals, whether or not treated with androgens, there was a considerable reduction in thickness of all three zones of the adrenal cortex, but no changes were evident within the cells of each zone.

Discussion. Failure of androgens to produce hypertensive vascular disease in hypophysectomized rats indicates that not only the adrenals (12, 13), but the pituitary is essential in the development of the disease. It is likely that ACTH stimulation is needed for the production of sufficient amounts of DOC by the adrenal glands of androgen-treated rats to cause hypertension. The importance of ACTH on the enhancement of MAD-induced hypertension has already been shown (8).

The renotropic and thymolytic effects of the androgens, already reported both in normal (14) and hypophysectomized (15) rats, suggest a direct action of these hormones on these target organs, independent of their effect on the adrenals and the pituitary. As previously shown (15, 16), the weights of the adrenals in hypophysectomized rats increased after androgen administration. In our experiment, however, such an increase was rather small and significant only in rats receiving testosterone. A significant increase in the weight of the ovaries was also found following MT or testosterone administration in contrast to that reported by Leonard (16). Our treatment, however, was more prolonged and that may explain such contradictory reports. It is remarkable that, contrary to the hypophysectomized rats, animals with an intact pituitary showed a severe reduction of both ovarian and adrenal weight following androgen treatment (9, 10), thus confirming the essential role of the pituitary gland in regulating some of the effects of these hormones.

Summary. Administration of methylandrostenediol, methyltestosterone and testosterone to hypophysectomized rats failed to produce the hypertensive vascular disease usually seen in normal animals receiving the same androgens. It is, therefore, evident that the pituitary, in addition to the adrenals, plays an essential role in the pathogenesis of androgen-induced hypertension and it is very likely that the constant stimulation of the adrenal gland by ACTH is essential to the development of such hypertensive disease.

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