The Effect of 5α-Dihydrocortisol on the Blood Pressure of Rats Treated with Deoxycorticosterone Acetate and Salt

Hiroshi Mikami, Charles A. Nugent*, Toshio Ogihara, Toru Naka, Keiichi Iwanaga and Yuichi Kumahara

Department of Medicine and Geriatrics, Osaka University Medical School, Fukushima-ku, Osaka, 553, Japan and *The Endocrine Section, Department of Internal Medicine, University of Arizona, College of Medicine, Tucson, Arizona 85724, USA

Abstract

5α-dihydrocortisol has been reported to amplify the mineralocorticoid activity of aldosterone. In this study 5α-dihydrocortisol was administered to unilaterally nephrectomized rats treated with 11-deoxycorticosterone acetate (DOCA) and sodium chloride to examine its potentiating effect on the elevation of blood pressure in these animals. Subcutaneous administration of DOCA at two dose levels (40 µg and 100 µg/100g of body weight 3 times a week) resulted in a significant rise in blood pressure when compared with controls given no DOCA. However, concomitant injection of 5α-dihydrocortisol (300 µg/100 g of body weight 3 times a week) with both doses of DOCA did not accelerate the development or potentiate the severity of the hypertension in a 4 week period. Furthermore, administration of 5α-dihydrocortisol did not cause a further decrease in plasma renin activity or a greater increase in urinary kallikrein excretion than those observed after DOCA alone. Thus, 5α-dihydrocortisol does not potentiate DOCA in the production of low renin hypertension in unilaterally nephrectomized salt-loaded rats.

The pathogenesis of low renin essential hypertension is unknown (Dunn and Tannen, 1977). Some investigators have presented evidence suggesting that the disorder does not exist as a separate disease entity (Dunn and Tannen, 1977). While considerable evidence has been developed to support the notion that hyporeninemia in some patients with essential hypertension is attributable to over-production of mineralocorticoids, the specific hormone or hormones responsible have not been identified (Liddle et al., 1973). Recently 5α-dihydrocortisol (5α-DHF) has been found in abnormally large amounts in the urine of a child with low renin hypertension (Ulick et al., 1977). This steroid has minimal mineralocorticoid activity when administered alone to rats, but when given with aldosterone it amplifies the effect of aldosterone on the urinary excretion of sodium and potassium (Adam et al., 1978). In this study we examined the potentiating effect of 5α-DHF on the hypertension produced in rats by salt loading and 11-deoxycorticosterone acetate (DOCA) administration.

Materials and Methods

DOCA (Sigma Chemicals Co., USA) and 5α-DHF (Makor Chemicals Ltd., Israel) were used without further purification. The steroids were

Received September 6, 1980.
prepared for injection by breaking the crystalline material into a fine suspension in sesame oil by vigorously agitating the suspension with glass beads 2-3 mm in diameter using a vortex mixer. Male Wister rats were fed food pellets (NaCl content 1.0% purchased from Oriental Yeast Co., Tokyo, Japan, and given drinking water containing 1% NaCl. One week before starting the study all rats underwent a left nephrectomy. The mean weight of the rats was 206 g (range 180 to 236 g) at the beginning of the study.

Forty-four rats were treated as shown in Table 1. The steroids were injected subcutaneously 3 times a week (Monday, Wednesday and Friday) in a small volume of sesame oil. The rationale for the selection of the steroids was as follows. Adam et al., (1978) reported that the mineralocorticoid effects of 1 µg of aldosterone were amplified by 60 µg of 5α-DOCA may have about 1/20 of the sodium-retaining activity of aldosterone (Muller et al., 1955). Therefore, for 20 µg of DOCA, an appropriate dose of 5α-DHF would be 60 µg. A moderate degree of hypertension can be induced in maturing male rats (unilaterally nephrectomized and salt loaded) within a month by injecting 100 µg of DOCA per 100 g of body weight subcutaneously 3 times a week. When larger doses of DOCA (400 to 500 µg/100 g each day) were given subcutaneously in oil into unilaterally nephrectomized salt-loaded rats, systolic blood pressure (BP) was two or more times higher than when the rats were given 100 µg/100 g 3 times a week. We selected 100 µg of DOCA with and without 300 µg of 5α-DHF for the treatment of rats (groups VI and V, respectively). To examine the effect of a higher dose of 5α-DHF relative to DOCA, we gave 40 µg of DOCA with and without 300 µg of 5α-DHF to 2 other groups of rats (IV and III, respectively). The remaining groups were given 200 µg of 5α-DHF with no DOCA (II) or no steroid (oil alone, I) 3 times a week. The mean volume of oil injected was 0.05 ml but the precise volume injected was dependent on the weight of the rat and ranged from about 0.04 to 0.06 ml.

The rats were kept in metabolic cages with two rats per cage placed in a temperature controlled room (25~27°C) illuminated between 6 a.m. and 8 p.m.. Injections were given over a 4 week period. Systolic BP was measured without anesthesia by tail plethysmography (USM-105-R, Ueda Seisakusho, Co., Tokyo, Japan) between 10 a.m. and 0 p.m. after prewarming the rats at 37°C for 10 minutes. BP and body weight were measured twice a week. Urine was collected on the 4th, 11th, 18th and 29th days for analysis of sodium, potassium and kallikrein. On the 30th day blood was obtained by cardiac puncture after light anesthesia for determination of hematocrit, serum sodium and potassium concentration and plasma renin activity (PRA). Electrolytes were measured with a Technicon autoanalyzer. PRA was determined by the method of Menard et al., (1972). Urine kallikrein activity was estimated by measuring proteolytic activity by the method of Morita et al., (1977). Analysis of variance was used to examine the effect of the six treatments on the BP, PRA and urinary kallikrein excretion. The significance of difference between the means for the control and the treated groups at each period of the experiment was examined by a method similar to Tukey’s but suitable for use when the number of animals in each group is not identical (Fukui et al., 1960).

Results

Rats injected with no steroids (oil alone, I) and those given 5α-DHF and no DOCA (II) showed no significant rise in BP during the 4 week study (Fig. 1). However, all rats given DOCA with and without 5α-DHF (III-IV) had a significant and persistent increase in BP above baseline values beginning 2 weeks after the start of the injections (Fig. 1). While the animals given the larger doses of DOCA tended to show higher BP than those given the smaller dose of DOCA, the difference was not statistically significant. The addition of 5α-DHF to both the large and small doses of DOCA had no significant amplifying effect on the DOCA produced hypertension.

Hematocrit and PRA were significantly lower in the groups treated with small and large doses of DOCA both with and without 5α-DHF (III-IV) than in the group treated with no steroids or with 5α-DHF and no DOCA (I and II, respectively).
Vol. 27, No.6  5α-DIHYDROCORTISOL AND BP  771

Fig. 1. The BS response of the rats to injection of different combinations of DOCA, 5α-DHF and oil are plotted as functions of time. Solid lines connect the values for mean BP in each group. Vertical lines express SEM. Asterisks indicate the significance of differences from the oil control group: * p <0.05, ** p <0.01.

Fig. 2. Mean PRA on day 30 of rats given oil (I), 5α-DHF (II), small DOCA (III), small DOCA+5α-DHF (VI). Vertical lines indicate SEM and asterisks indicate the significance of differences from the oil control group: *** p <0.001.

Only the data for PRA are shown in detail (Fig. 2). Serum sodium and potassium concentrations and the amounts of these electrolytes excreted in the urine in all groups of rats did not differ significantly from each other.

Urinary kallikrein excretion (Fig. 3) increased throughout the study in groups I and II in association with the increasing body weight (mean gain of 119 g in 4 weeks). However, after the first week of the study, the groups treated with DOCA (III-VI) showed increasingly elevated urinary kallikrein excretions above those seen in groups I and II but the differences were significant only in the last week. There was no significant differences in urinary kallikrein excretion between groups III and
IV and between V and VI throughout the study.

Discussion

Several steroids are reported to amplify the mineralocorticoid activity of aldosterone and in this way might be involved in causing low renin hypertension. Dale and Melby (1974) found that 16α, 18-dihydroxy-DOC potentiated the in vivo sodium-retaining activity of aldosterone. However, Fuller et al. (1976) were unable to confirm this result. Sekihara et al. (1976) found that 19-ol-androst-4-ene-3, 17-dione amplified the activity of aldosterone in a bioassay system using adrenalectomized rats.

New et al. (1977) reported a patient with juvenile hypertension, suppressed PRA and hypokalemic alkalosis without evidence of over-production of aldosterone or other known mineralocorticoids. In their intensive search for steroids that might be responsible for the abnormal findings in their patient they found an unusual increase in dihydro-metabolites of cortisol, especially 5α-DHF (Ulick et al., 1977). Marver and Edelman (1978) reported that 5α-DHF bound with a high affinity to aldosterone receptors in rat kidney and stimulated sodium transport in the isolated toad urinary bladder. These investigators suggested that this steroid might be responsible for the abnormalities reported by Ulick et al. (1977) in their patient. However, Adam et al. (1978) and more recently Sekihara et al. (1978) found that 5α-DHF had minimal, if any, mineralocorticoid activity in a bioassay system using the changes in urinary electrolytes after injection of the steroid in adrenalectomized rats as an index of mineralocorticoid activity. After further investigation of the problem Adam et al. (1978) found that 5α-DHF potentiated mineralocorticoid activity of aldosterone when they were administered together. They also found that the steroid had a low affinity with mineralocorticoid receptors in vitro. These results suggested that amplification of the mineralocorticoid activity of aldosterone by 5α-DHF might be through an unusual mechanism.

In our study we used rats with mineralocorticoid hypertension to examine the possibility that 5α-DHF might amplify the BP elevation. A dose of 300 μg of 5α-DHF per 100 g of body weight 3 times a week caused no significant rise in the BP of heminephrectomized rats drinking 1% saline (II vs. I). Furthermore, this dose of 5α-DHF did not accelerate the development or potentiate the severity of the hypertension produced by injection of 100 μg of DOCA/100 g of body weight (VI vs. V). This dose of 5α-DHF relative to the amount of mineralocorticoid administered was an amount which in vitro studies had led us to expect to be adequate for amplification of mineralocorticoid activity. When the ratio of 5α-DHF to DOCA was increased by a factor of 2.5 there was also no indication of any amplifying effect of 5α-DHF on mineralocorticoid activity (IV vs. III).

Mineralocorticoids suppress PRA (Pettinger et al., 1971) and increase urinary kallikrein excretion in rats (Margolius et al., 1972). Our animals given DOCA with and without 5α-DHF had suppressed PRA and increased kallikrein excretion. These changes were not significantly different between groups given 5α-DHF plus DOCA and the groups given DOCA alone.

Recently, Ulick et al. (1979) restudied their original patient with juvenile hypertension and reported that her hypertension and hypokalemic alkalosis persisted while her urinary 5α-DHF became undetectable as she grew older. The features of the patient's disease suggesting a hypermineralocorticoid state can no longer be attributable to 5α-DHF overproduction unless, as suggested by Ulick et al. (1979), some metacorticoid mechanisms were postulated. Our results provide no support for the postulate that...
5α-DHF can potentiate mineralocorticoids in the production of hypertension. It is unlikely that 5α-DHF is responsible for low renin essential hypertension in man.

Acknowledgements

This work was supported in part by a grant from the Ministry of Education and the Ministry of Health and Welfare ("Diorders of Steroid Hormones" Research Committee), Japan.

References