Evidence of Abnormalities in Corticosteroid Secretion Leading to Volume-Dependent Hypertension in Milan Rats

Robert Fraser, Aidan K. Ancil, William B. Brown, Mary C. Ingram, Christine D. Holloway, Ian W. Henderson, Christopher J. Kenyon

Abstract We examined corticosteroid secretory patterns and their relation to altered salt and water metabolism in Milan hypertensive and normotensive rats. Hypertensive rats had significantly higher blood pressures, exchangeable sodium (hypertensive, 41.2±0.3 mmol · kg⁻¹; normotensive, 38.4±0.03 mmol · kg⁻¹, P<.001), plasma volume (hypertensive, 5.39±0.12 mL · 100 g⁻¹; normotensive, 4.84±0.10 mL · 100 g⁻¹, P<.001), and plasma concentrations of atrial natriuretic peptide (hypertensive, 38.8±4.0 pg · mL⁻¹, normotensive, 22.4±3.1 pg · mL⁻¹, P<.02). These features coincide with those of mineralocorticoid-induced hypertension. Adrenal venous secretory rates (picomoles per minute) of corticosterone (hypertensive, 1696±202; normotensive, 873±139), 18-hydroxy-corticosterone (hypertensive, 49.7±8.3; normotensive, 25.7±3.3), and aldosterone (hypertensive, 1.16±0.17; normotensive, 0.52±0.08) were higher in the hypertensive than the normotensive strain, but that of 11-deoxycorticosterone (DOC) (hypertensive, 94.4±14.9; normotensive, 114.3±33.9) was similar in the two strains. The corticosterone-DOC, 18-hydroxy-corticosterone-DOC, and aldosterone-DOC ratios were higher in the hypertensive than the normotensive strain (P<.02), but the 18-hydroxy-corticosterone–corticosterone and aldosterone–18-hydroxy-corticosterone ratios were not. These results indicate increased activity of the "late" aldosterone biosynthetic pathway in the hypertensive compared with the normotensive strain caused by an increased conversion rate of DOC to corticosterone. The comparison of corticosteroid secretion between the two strains indicates that 11β-hydroxylase rather than aldosterone synthase activity is more active in the hypertensive than the normotensive rats. (Hypertension. 1994;24:512-515.)

Key Words • adrenal gland hyperfunction • adrenal cortex hormones • corticosterone • aldosterone • steroid 11-hydroxylase • plasma volume • rats, inbred SHR

Several inbred rat strains with a genetically determined tendency to develop hypertension have been used as models for human essential hypertension.¹ Cosegregating physiological, hormonal, and biochemical features vary among models. Study of the Milan hypertensive (MHS) rat has the advantage of the availability of a simultaneously selected normotensive control population (MNS rats).²-⁴ MHS rats have smaller kidneys than MNS rats⁵ but similar numbers of nephrons.⁶ Glomerular filtration rate is higher per unit weight of kidney in MHS rats.⁶,⁷ Transplantation of kidneys from MHS to MNS rats results in hypertension in the recipients.⁸ In MHS rats both erythrocyte and proximal tubular cells (but not distal tubular cells) are smaller than in MNS rats.⁹ The erythrocyte Na⁺-K⁺ cotransport rate is higher in MHS rats, a trait that cosegregates with hypertension in breeding studies.⁵,⁹ It has therefore been concluded that an inherited defect of proximal tubular cell ion transport, possibly caused by a cell membrane abnormality, is partly responsible for the onset of hypertension.

Interestingly, MHS rats also have significantly higher plasma corticosterone concentrations than MNS rats throughout life, and aldosterone excretory rates are higher in adult hypertensive rats.⁵,¹⁰,¹¹ Adrenal weight is also higher in MHS rats.¹¹ The causes of these differences are not known, but it is relevant that both mineralocorticoids and glucocorticoids are known to influence many of the phenotypic differences between MHS and MNS rats, including blood pressure and renal and extrarenal cation transport (reviewed in Reference 12). This study attempts to identify defects in corticosteroid biosynthesis and to relate them to changes in electrolyte and water metabolism.

Methods

Animals

Male rats were from colonies of MHS and MNS rats established at the University of Sheffield from breeding pairs imported from Milan. Experimental procedures complied with the regulations of the British Home Office animal licensing authority.

Plasma Volume and Exchangeable Body Sodium

Exchangeable sodium was measured in groups of eight MHS and MNS rats from the age of 6 to 13 weeks by the method of McAreevey et al.¹³ Briefly, rats were fed a diet with negligible amounts of sodium (<20 pmol/kg) and drank a sodium chloride solution (85 mmol · L⁻¹) containing ²²Na (37 Kβq · L⁻¹). After 2 weeks of equilibration, total body radioactivity was measured at weekly intervals using an Armac scintillation spectrometer (Packard Instruments). Blood pressure was measured in warmed conscious rats by tail plethys-
mography using a W&J recorder. After a washout period, plasma volume was measured by dye dilution using Evans blue. Samples for atrial natriuretic peptide (ANP) analysis were collected at this time (20 weeks).

**Adrenal Venous Blood Collection**

With rats under thiobutabarbital anesthesia (Inactin, Byk-Gulden; 100 mg/kg body wt), a catheter was placed in the left jugular vein of adult (approximately 250 g) rats, and an infusion of physiological saline (700 µL·min⁻¹·kg body wt⁻¹) was begun. After approximately 5 minutes, a laparotomy was performed and the animal heparinized (100 U·kg body wt⁻¹). The left adrenal vein was ligated at its point of entry into the renal vein and punctured with a siliconized needle (25 gauge) attached to a catheter. Adrenal venous blood flowed freely into chilled collection vessels for up to 45 minutes. Plasma was separated and stored at -20°C until analysis.

**Hormone Assays**

Plasma hormone concentrations were measured by radioimmunoassay. ANP was extracted with C18 cartridges (Sep-Pak, Millipore Corp) before assay. Corticosteroids were extracted and partially purified by paper chromatography.

Results are expressed as mean±SEM; comparisons were made by Student's t test.

**Results**

**Blood Pressure and Electrolytes**

Higher blood pressure in 20-week-old MHS rats was associated with increased plasma volume relative to MNS rats (Fig 1). At this time, plasma ANP concentrations were also significantly raised. Exchangeable sodium was higher in MHS than MNS rats from 10 weeks of age (data not shown). The values shown for exchangeable sodium in Fig 1 are measurements made at 20 weeks. *P<.02, **P<.001.

**Corticosteroid Secretion Rates**

Adrenal venous flow rates (microliters per minute) were similar in MHS and MNS rats (MHS, 97±8, n=17; MNS, 81±9, n=14). Secretion rates of corticosterone, 18-hydroxy corticosterone (18-OHB), and aldosterone were all significantly higher in MHS than MNS rats (Fig 2); that of 11-deoxycorticosterone (DOC) was not significantly different between strains. Fig 3 shows secretion rate ratios for the various components of the late aldosterone pathway. The corticosterone-DOC, 18-OHB-DOC, and aldosterone-DOC ratios were significantly higher in MHS than MNS rats, but the 18-OHB-
Higher urinary aldosterone secretion rates\(^1\)\(^-\)\(^4\) and hypertrophy of the zona glomerulosa with more active mitochondria and reduced lipid content have been described.\(^1\) Carrere, an anti-mineralocorticoid, prevents the rise in blood pressure in MHS rats, although this effect has been attributed to antagonism to ouabain-like factors.\(^2\) More striking, however, are repeated findings of raised plasma corticosterone concentrations throughout life.\(^1\)\(^1\)\(^1\)\(^1\)\(^1\) Although corticosterone is recognized as the principal glucocorticoid hormone in the rat, it has been demonstrated recently that, were it not for the inactivating enzyme 11\(\beta\)-hydroxysteroid dehydrogenase (11\(\beta\)-OHSD), corticosterone would bind readily to renal mineralocorticoid receptors.\(^2\) Indeed, Stewart et al\(^4\) have investigated whether plasma corticosterone is elevated because of an impairment in the gene encoding 11\(\beta\)-OHSD in MHS rats. No difference in renal 11\(\beta\)-OHSD activity was found between strains, but hepatic activity was lower in MHS rats. So although renal steroid metabolism is unlikely to account for mineralocorticoid excess, it could partly explain higher circulating hormone levels.

To measure adrenocortical secretions without the confounding influence of different metabolism rates, we directly measured adrenal vein secretion rates. Because of the effects of anesthetics and frequency of sampling, adrenocortical function is likely to have been maximally stimulated in the present study so that secretory rates will reflect steroidogenic capacity. Aldosterone, 18-OHB, and corticosterone but not DOC secretion rates were greater from MHS than MNS rat adrenals. Bearing in mind that secretions of zona glomerulosa and inner zones are mixed in adrenal venous blood, these results indicate that adrenocortical function is affected generally. Increased output of aldosterone and 18-OHB, which are products of the zona glomerulosa-specific enzyme aldosterone synthase, is in agreement with previous observations of greater metabolic activity in this area of the gland. Corticosterone, although produced by all zones of the rat adrenal, is secreted in this area of the gland. Our observation of increased corticosterone secretion by MHS rat adrenals is in line with in vivo observations of raised plasma corticosterone concentrations in quiescent rats but does not appear to be associated with a change in zona fasciculata metabolic activity.\(^1\)

One possible explanation of MHS rat steroidogenesis is that an enzyme complex to both zona glomerulosa and zona fasciculata is affected. Closer analysis of the ratios of precursor and products of the final steps in aldosterone and corticosterone biosynthesis suggest that 11\(\beta\)-hydroxylase is more active in MHS rats. However, this straightforward analysis is complicated by recent findings in humans and rats of two enzymes, 11\(\beta\)-hydroxylase (cytochrome P-450\(^{11\beta}\)) and aldosterone synthase (cytochrome P-450\(^{18\alpha}\)), that catalyze the conversion of deoxycorticosterone to corticosterone and are expressed in the zona glomerulosa.\(^4\)\(^-\)\(^6\) It is important to state here that the quiescent zona glomerulosa of the Wistar rat does not express the 11\(\beta\)-hydroxylase gene although low-level expression follows corticotropin stimulation. Whether this is true also for Milan rat adrenal glands is not known. Only aldosterone synthase is thought to be regulated by dietary sodium manipulations, so it has been assumed that 11\(\beta\)-hydroxylase is not involved in aldosterone biosynthesis.\(^6\)\(^-\)\(^7\) The present results indicate that this may not invariably be the case. Either 11\(\beta\)-hydroxylase plays a more important part in the physiological control of aldosterone synthesis than hitherto realized, or our observations are an artifact produced by a hyperstimulated gland. Whatever the explanation, there appears to be a phenotypic difference in 11\(\beta\)-hydroxylase activity between MHS and MNS rats.

The same enzyme is one of several genetic factors that influence the development of hypertension in Dahl rats.\(^8\) Recently, several mutations in the 11\(\beta\)-hydroxylase gene of salt-resistant rats have been linked to altered steroid metabolism; these mutations in turn cosegregate with low blood pressure in crosses of Dahl salt-sensitive and salt-resistant rats.\(^9\)\(^-\)\(^10\) The nature of the differences in steroid metabolism between salt-sensitive and -resistant rats is not the same as that between MHS and MNS rats. 11\(\beta\)-Hydroxylase has the capacity to hydroxylate corticosteroids at positions 18 and 19 as well as 11\(\beta\).\(^11\)\(^-\)\(^12\) The 11\(\beta\)-hydroxylase gene of salt-sensitive Dahl rats favors 18- rather and 11-hydroxylated products. Although this is not the same phenotype as that of MHS rats, it does not exclude the possible involvement of MHS 11\(\beta\)-hydroxylase in the development of hypertension. It should be remembered that this enzyme holds a pivotal position in steroid biosynthesis between three or four potent hypertensinogenic steroids. The balance between DOC, aldosterone, corticosterone, and 19-nordeoxycorticosterone is controlled by the activity or selectivity of this enzyme.

One further point of evidence to support our hypothesis that increased 11\(\beta\)-hydroxylase activity causes hypermineralocorticoidism and possibly influences blood pressure comes from our recent collaborative genetic studies. A polymorphism in the 11\(\beta\)-hydroxylase gene of MHS and MNS rats has been identified. In breeding studies the MHS 11\(\beta\)-hydroxylase genotype cosegregates with a secondary marker of mineralocorticoid activity, urine volume. Although the influence of this gene alone on blood pressure is weak, there appears to be an epistatic link with other factors (C.J.K. et al, unpublished observations, 1994).

In summary, corticosteroid biosynthesis is altered in MHS rats compared with MNS rats and this may be caused by a difference in 11\(\beta\)-hydroxylation. In hypertensive rats, this might be one of several underlying causes of apparent mineralocorticoid excess that we have observed and that may determine blood pressure differences compared with normotensive controls.

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