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SHORT COMMUNICATION

RENAL 11β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY: EFFECTS OF AGE, SEX AND ALTERED HORMONAL STATUS

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Summary—The enzyme 11β-hydroxysteroid dehydrogenase, by converting cortisol and corticosterone to their receptor-inactive 11-keto metabolites cortisone and 11-dehydrocorticosterone, appears crucial to the aldosterone-selectivity of renal mineralocorticoid receptors. Levels of enzyme activity in the rat kidney, measured by conversion of cortisol to cortisone, are unaltered by changes in adrenal or thyroid status, or by castration in either sex; in contrast, oestrogen administration increases enzyme activity in male rats.

INTRODUCTION

In 1977 Ulick *et al.* first described the syndrome of apparent mineralocorticoid excess, characterized by hypertension and Na^+ retention, low plasma renin and aldosterone, and normal or subnormal plasma cortisol levels [1]. Over the subsequent decade the underlying abnormality in steroid metabolism has been established to be very low activity of the enzyme 11β-hydroxysteroid dehydrogenase (11β-DH), responsible for the conversion of cortisol to its inactive metabolite cortisone, and reflected in grossly elevated urinary ratios of cortisol to cortisone metabolites [2, 3].

The state of obvious mineralocorticoid excess, and the response of such patients to spironolactone despite the low levels of known mineralocorticoids, prompted an initial search for an unidentified mineralocorticoid which might be responsible for the syndrome. When such a factor could not be identified, attention turned to the receptor, and the possibility that cortisol might be inappropriately active as a mineralocorticoid was suggested [2]. The physiological importance of renal dehydrogenation of cortisol was first emphasized by the studies of Edwards and his colleagues [4], and later confirmed by them in an elegant study of the affects of liquorice ingestion on Na^+ status and cortisol clearance [5].

This focus has been extended recently by three series of experimental studies in the rat, in which the physiological role of 11β-DH in conferring aldosterone-selectivity on otherwise non-selective type I (mineralocorticoid) receptors has been proposed. We have shown that carbenoxolone Na^+ will block this enzyme in kidney, colon and parotid, and under the same circumstances will largely (colon) or completely (kidney, parotid) abolish the aldosterone selectivity of mineralocorticoid receptors *in vivo* [6]. In parallel studies, Edwards *et al.* showed that rat kidney slices pretreated with glycyrrhetinic acid bound [^{3}H]corticosterone extensively in the inner cortical region: untreated slices showed minimal binding [7]. Finally, Souness and Morris have shown that corticosterone, at doses which have no mineralocorticoid effect when given alone, markedly affects urinary Na^+/K^+ ratios in the presence of carbenoxolone [8].

Given the apparent key role thus played by this enzyme in conferring aldosterone selectivity on renal mineralocorticoid receptors, we determined to extend the previous studies [9] on control of activity of the enzyme in the kidney. Whereas the previous reports have focussed on changes with ontogeny, the present studies explore the effects of age, sex, and adrenal and thyroid status on enzyme activity.

MATERIALS AND METHODS

[1,2-^{3}H]cortisol (50 Ci/mmol) and [1,2-^{3}H]cortisone (20 Ci/mmol) were from Amersham (Buckinghamshire, England), unlabelled cortisol and cortisone from Sigma Chemical Company (St Louis, Mo., U.S.A.), thyroxine sodium (T4) as Oroxine tablets from Wellcome (Sydney, Australia), propylthiouracil (PTU) from Cilag (Sydney, Australia), dexamethasone Na^+ phosphate from David Bull Laboratories (Melbourne, Australia) and oestradiol (E_2) and dihydrotestosterone (DHT) from Sigma.

Experiments were performed on male and female Sprague–Dawley rats (Central Animal House, Monash University, Australia). All animals were maintained on a 12 h light:dark cycle (lights on 0600 h), and were allowed free access to food and water.

Bilateral adrenalectomy (ADX) was performed under ether anaesthesia; rats were thereafter given 0.9% NaCl to drink. Dexamethasone was commenced on day 4 post-adrenalectomy (1 mg/l drinking fluid) for 3 days. Animals given PTU and/or T4 were treated for 21 days (100 mg PTU/l of drinking water, 10 μg T4/100 g body wt by intramuscular injection); in all rats PTU administration was verified by inspection of the thyroid gland at sacrifice, and T4 and PTU administration confirmed by measurement of plasma T3 and T4. In control female rats, T3 and T4 levels were 1.8 ± 0.1 and 58 ± 5 nmol/l respectively; in T4-treated females 3.0 ± 0.3 and 102 ± 9; in the PTU-treated group 0.9 ± 0.1 and < 10; and in the group receiving both T4 and PTU, 1.5 ± 0.1 and 145 ± 9. For male rats, control values were 1.6 ± 0.1 and 69 ± 2 nmol/l: in the T4-treated group 3.4 ± 0.2 and 98 ± 5; in PTU-treated males 0.9 ± 0.1 and

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< 10; and in the PTU/T4-treated group, 1.3 ± 0.1 and 263 ± 9. Rats were castrated under ether anaesthesia 8 days prior to use, and given 100 μg DHT or 6 μg E_2 in oil by intramuscular injection daily.

Rats were decapitated, and kidneys removed and immediately placed in 0.9% saline on ice. Tissues were minced by hand with scalpel blades, and 0.5 g of minced tissue incubated for 1 h at 37°C in 2 ml saline containing approximately 200,000 cpm tritiated steroid (approximately 3 nM final conc.). In preliminary studies ($n = 4$) we showed equilibrium to have been reached by 1 h, with plateau levels of [^{3}H]cortisone not different between 1 and 2 h of incubation. Reactions were terminated by the addition of 5 vol ethyl acetate, and the vials shaken vigorously to extract the steroids into the ethyl acetate. The aqueous phase was frozen, and the organic phase decanted and evaporated to dryness under air. The steroid residue was then dissolved in 0.1 ml ethanol, carrier steroids in ethanol added, and the mixture diluted to 0.5 ml with 0.1% trifluoroacetic acid (TFA) for high-performance liquid chromatography (HPLC) analysis. Retention times of authentic standards were established prior to injection of samples.

Tritiated samples were injected onto a radially compressed CN reversed-phase column and eluted isocratically with 16% acetonitrile for cortisol, and 19% acetonitrile for corticosterone, plus 0.08% TFA at 1 ml/min. Fractions (0.5 ml) were collected over 20 min and 4 ml scintillation fluid (Emulsifier-safe, Canberra Packard, Downers Grove, Il.) added for measurement of radioactivity as counts per minute per fraction by liquid scintillation spectrometry.

Controls comprised (i) a zero time incubation, in which saline containing tritiated steroid was added to the tissue, ethyl acetate added immediately and the vial frozen (T_0), and (ii) a blank vial to which saline containing tritiated steroid was added without tissue, prepared at the same time as the sample vials and incubated in parallel with them. Both sets of controls showed negligible conversion of cortisol (F) to cortisone (E) or of E to F.

Percent conversion of F to E was calculated by adding the counts per minute in each tritiated peak, and then dividing: total E cpm/total cpm of all peaks $\times$ 100.

Statistical analyses were by either Student's t-test or one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

The possibility of difference with age and sex in renal dehydrogenase activity was studied by comparing the metabolism of cortisol in male and female rats, at weaning (approx. 20 days of age) and at sexual maturity (approx. 60 days of age). Figure 1 shows the percent conversion of [^{3}H]F to [^{3}H]E in male and female rats at 23 days of age (panel A) and 65 days of age (panel B). At 23 days of age there is no significant difference between male and female rats. In mature rats, however, there is greater conversion of [^{3}H]F to [^{3}H]E in males than in female rats after 2 h albeit under suboptimal conditions to compare the rate of conversion. These data are shown, however, to illustrate the third, later eluting peak prominent only in mature male rats (panel C).

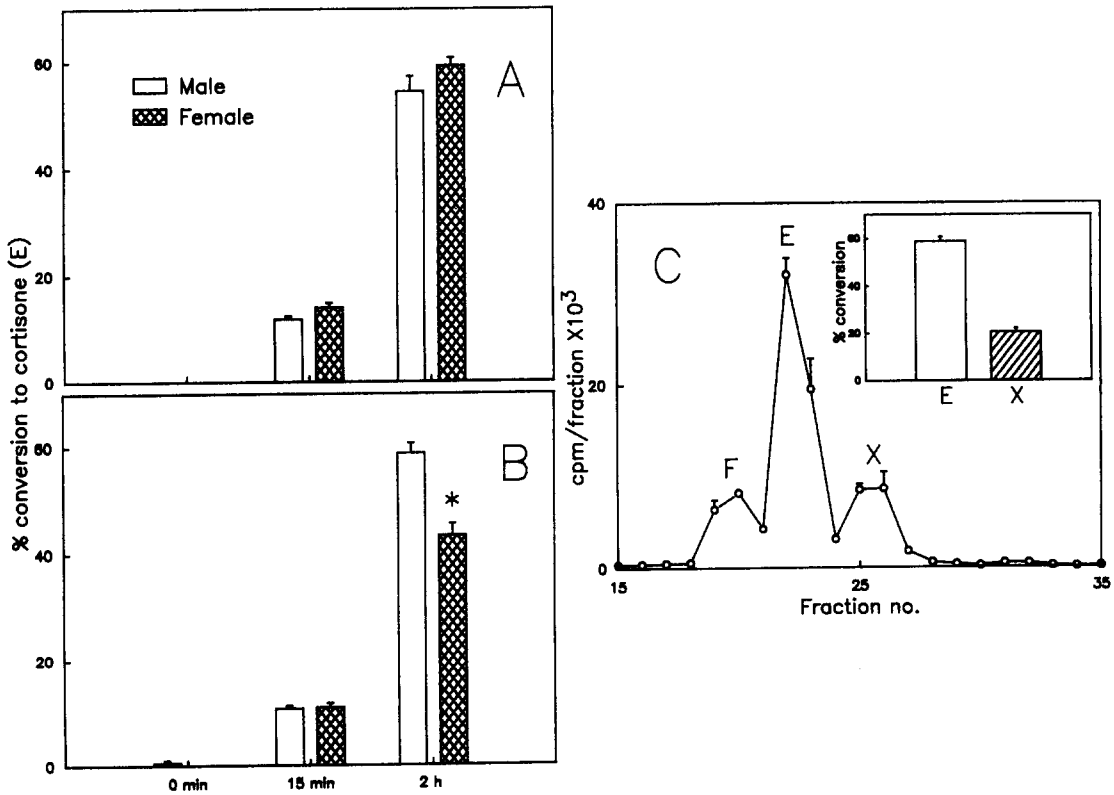

Fig. 1. (A and B) Percent conversion of [^{3}H]cortisol (F) to [^{3}H]cortisone (E) in both male and female rats at 23 days of age (panel A) and 65 days of age (panel B) at 0, 15 min and 2 h incubation. (C) HPLC profile obtained from 2 h incubation of 65 day old male kidney minces with [^{3}H]cortisol. Peak 1 represents the elution position of authentic cortisol (F), peak 2, cortisone (E) and peak 3, X. Insert illustrates the conversion of [^{3}H]F to both [^{3}H]E and [^{3}H]X. Values are expressed as the mean $\pm$ SEM ($n = 4$ rats per group). Mature male rats showed significantly higher conversion to [^{3}H]E than mature female rats ($P < 0.05$) by Student's t-test.

Table 1. Percent conversion of cortisol (F) to cortisone (E) in male and female rat kidney

Intact	Intact + DM	ADX	ADX + DM
♂38.0, 38.0	37.0, 38.0	37.0, 38.0	39.0, 40.0
Control	PTU	T4	PTU/T4
♂24.5 ± 1.5	18.0 ± 1.5	20.3 ± 1.5	20.7 ± 2.6
♀20.5 ± 3.5	22.0 ± 2.0	16.0 ± 2.5	16.7 ± 2.9
Intact	E_2	Castrate	Castrate + DHT
♂39.8 ± 2.3	53.0 ± 1.5*	33.6 ± 1.7	31.4 ± 2.6
Intact	E_2	Castrate	Ovx + E_2
♀18.3 ± 1.5	22.3 ± 0.9	16.5 ± 1.0	16.0 ± 1.8

Data are individual values, $n = 2$ (ADX/DX study), mean ± SEM, $n = 3$ (PTU/T4 studies) or $n = 4$ (E_2/DHT studies). *$P < 0.01$ (ANOVA) compared with intact control.

The insert shows at 2 h incubation the percent conversion to both E and this as yet unidentified metabolite (X).

The possibility that 11β-DH activity may be modulated by changes in glucocorticoid, thyroid and gonadal hormone status was studied in a series of ablation and replacement experiments in mature male and female rats. As shown in Table 1, there appears to be no significant effect of adrenalectomy, or of dexamethasone administration to intact or adrenalectomized animals. Similarly, there are no significant effects of alteration of thyroid hormone status on 11β-DH activity (or levels of metabolite X) in either males or females measured in three separate pools of rat kidneys (Table 1).

In contrast, intact male rats injected with oestradiol show an increase in renal 11β-DH activity. Though overall levels of activity are higher in this group of rats than in those used for the T4/PTU study, this further increase is clearly not due to a chemical castration effect of oestrogen, via pituitary feedback to suppress LH and thus testosterone, since in castrate male rats mean levels were no higher than control. Not surprisingly, castrate male levels were not affected by DHT administration. The apparent oestrogen dependence of conversion of F to E was further investigated in a series of studies on young mature female rats (49 days old). These rats showed patterns of conversion essentially unaffected by either oestrogen administration or ovariectomy. The identity of peak X has not been further pursued in this present study.

There appear to be two major areas of discussion of the results presented above. First, there is sometimes considerable variation between individual studies in the activity of the 11β-DH, as measured by conversion of [^{3}H]F to [^{3}H]E. One example which might be cited is the difference in control conversion shown in Table 1, in which male rats of similar age showed much more extensive conversion in the E_2/DHT and ADX/DM studies than in the PTU/T4 study. The extent to which this variation is biological or technical is difficult to determine: the relatively low level of variation within each group and study suggests that it reflects real differences in activity of dehydrogenase, reductase and other enzymes (or required cofactors) rather than experimental imprecision. Finally, the variability observed between individual studies emphasizes the need for controls within each experiment rather than historical controls, even when animals are matched for age and sex.

The second area for discussion is that of the apparent insensitivity of levels of 11β-DH activity to pertubations of the pituitary–adrenal, thyroidal or gonadal axes. One warning that needs to be sounded is that all of the ablation and replacement studies were relatively acute (ADX ± DM 4 days; castration ± replacement 8 days; PTU ± T4 21 days), so that possible changes requiring a more prolonged withdrawal of hormones may not be seen. In terms of the adrenal steroids, for example, there is a wide variety of acute responses, for both mineralocorticoids and glucocorticoids: in contrast, however, the effects of intracerebroventricular aldosterone on blood pressure take 2 weeks to become manifest [10]. In studies on humans, Hellman *et al.* [11] showed that administration of T3 for 10–21 days produced modest elevation of THE to THF ratios, consistent with an increased activity of 11β-DH; in contrast, *in vitro* studies on rat liver by McGuire and Tompkins [12] showed the predominant effect of thyroid hormone to be on 5α reduction, with no increase in 11-ketosteroid formation, consistent with our present studies. This caveat notwithstanding, we believe that the present data can be reasonably interpreted as evidence against the total renal levels of 11β-DH activity being acutely responsive to signals from the adrenals, thyroid or gonads.

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