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NOTE

Increased ACTH Levels Do not Alter Renal 11β-Hydroxysteroid Dehydrogenase Type 2 Gene Expression in the Sheep

ANTHONY L. ALBISTON, DUANA MATSACOS AND JOHN MCDOUGALL

Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, 3052, Victoria, Australia

Abstract. The regulation of renal 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) gene expression is poorly understood. Inhibition of expression can result in hypertension. An example of this is in ectopic adrenocorticotropin (ACTH) syndrome (EAS). Inhibition of 11βHSD2 activity is suggested by the observed increased ratio of cortisol to cortisone in both plasma and urine. To investigate whether ACTH or ACTH-dependent steroids can modulate renal 11βHSD2 gene expression we analysed renal 11βHSD2 mRNA levels after treatment with ACTH of 1 H and 24 H and demonstrated no change in the levels of gene expression. We have demonstrated in this study that the expression of 11βHSD2 in the kidney is unaltered by ACTH. The reduced inactivation of cortisol by 11βHSD2 observed in EAS is likely to be in part due to end product inhibition or substrate overload of the enzyme by endogenous substrates (cortisol, corticosterone, etc) rather than inhibition of 11βHSD2 at the transcriptional level by either ACTH or ACTH regulated steroids.

Key words: 11βHSD2, Ectopic ACTH syndrome, Kidney, Hypertension

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In vitro studies have demonstrated that the mineralocorticoid receptor (MR) has equal affinity for aldosterone and the glucocorticoids, cortisol and corticosterone [1, 2]. Although glucocorticoids are present in the circulation at 100–1000 fold higher levels than aldosterone, under normal physiological conditions they do not bind to the MR. The aldosterone specificity of the MR, in aldosterone target tissues, is maintained by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), which converts glucocorticoids to their receptor inactive metabolites [3, 4].

Inhibition of 11βHSD2 activity enables glucocorticoids to inappropriately bind to the MR and results in hypertension as illustrated by glycyrrhetinic acid (liquorice) abuse [5] and the congenital syndrome of Apparent Mineralocorticoid Excess (AME) [6]. AME is a recessive disorder that is characterised by severe hypertension, hypokalemia and low plasma renin activity. Mutations in the 11βHSD2 gene have been determined in nearly 50 AME cases to date and was the third single-gene defect causing hypertension identified [7, 8]. Impaired enzyme activity has also been observed in some patients with essential hypertension [9] and in patients with ectopic ACTH syndrome [10].

Deficiency of 11βHSD2 in AME type 1 and in the ectopic ACTH syndrome is associated with an increased ratio of cortisol to cortisone in plasma, an increased ratio of the urinary metabolites of cortisol relative to cortisone and an increased half life for [11α-^{3}H]cortisol [10]. However in essential hypertension and AME type 2 the plasma and urinary ratios are unaffected and only the [11α-^{3}H]cortisol half life is altered [9].

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Correspondence to: Dr. A.L. Albiston, Howard Florey Institute University of Melbourne, Parkville VIC 3010 Australia

Unlike Cushing's syndrome ectopic ACTH syndrome (EAS) is characterised by symptoms of mineralocorticoid excess, including hypokalemic alkalosis and decreased plasma renin levels. In EAS the ratio of cortisol to cortisone in both plasma and urine is decreased indicating a decrease in renal 11βHSD2 activity due to ACTH or by ACTH-dependent steroids [10]. The increase in the cortisol and cortisone ratio in both plasma and urine is observed in humans also during ACTH infusion, but not following cortsiol infusion [10, 12]. Thus the inhibitory effect of ACTH on renal 11βHSD2 appears not to be mediated by increased cortisol levels, but either directly by ACTH or ACTH-dependent steroids other than cortisol. We postulated that the effect of ACTH on renal 11βHSD2 activity may in part be at the level of gene transcription. We have therefore investigated the effect of ACTH infusion on renal 11βHSD2 gene expression in the sheep.

We have chosen the sheep as our model of investigation as it has a similar adrenalcortical profile of steroid production as human eg the primary glucocorticoid is cortisol unlike rodents where the predominant glucocorticoid is corticosterone. This is an important consideration when investigating factors affecting 11βHSD2 as the enzyme, in all species studied to date has approximately an order of magnitude greater affinity for corticosterone compared with cortisol [13, 14].

Materials and Methods

Animals

Adult cross-bred Merino ewes were treated with ACTH (synacthen 1-24) 0.5 μg/kg/hr for 1 or 24 h, control animals were treated with saline for 24 h. Each groups had an n=4 and samples were individually analysed for both mRNA and enzyme activity levels. Animals were sacrificed with an intravenous bolus dose of sodium barbitone (Lethobarb, Arnolds. Reading, UK.). Kidneys were collected and immediately snap frozen in liquid nitrogen.

To confirm the efficacy of the ACTH treatment plasma cortisol levels were determined for samples taken before treatment and before sacrifice and are given as mean +/− standard error.

mRNA Quantitation

Total RNA was extracted from sheep kidneys using the method of Chirgwin *et al.* (1979) [15]. Levels of 11βHSD2 mRNA and Glyceraldehyde Phosphate Dehydrogenase (GAPDH) mRNA determined as an (internal control) were determined using a quantitative S1 nuclease protection assay. This method has been described previously [16]. [α-^{32}P]UTP (Dupont, Boston, Ma, USA) labelled antisense RNA probes were synthesised using the Promega Riboprobe kit (Promega, Sydney, Australia). The ovine GAPDH probe has previously been described [16] and the ovine 11βHSD2 probe was generated from a PCR product corresponding to nucleotides 396–892 of the published sequence and subcloned into a KS vector [17]. Results given as mean +/− standard error, standardized against GAPDH mRNA levels. Statistical analysis was by one-way analysis of variance.

11βHSD2 Activity Assay

11βHSD2 activity was determined as previously described [14]. Kidney samples were homogenised in 10 mM sodium phosphate buffer (pH 7.4), 0.25 M sucrose, 1 mM PMSF. Duplicate assay tubes containing 100,000 cpm of [1,2,67-^{3}H(N)]cortisol (Amersham International, Sydney Australia), 0.1 μM Cortisol, 0.5 mM NAD in 10 mM $NaPO_4$ buffer (pH 7.4) were pre-incubated for 10 min @ 37 C before the addition of 10 μg of protein, giving a final volume of 500 μl. Samples were incubated for 15 min @ 37 C followed by steroid extraction with ethyl acetate. Steroids were separated using thin layer chromatography in the solvent system chloroform: ethanol, 92 : 8. Results given as mean +/− standard error, percent conversion of cortisol per μg of protein. Statistical analysis was by one-way analysis of variance.

Results and Discussion

Serum levels of cortisol were shown to increase significantly in all sheep treated with ACTH; In the one hour treatment plasma levels of cortisol were 316 +/− 20 nM and with the 24 h treatment the levels rose to 398 +/− 63 nM. This is an increase of approximately three-four fold over maximal diurnal

levels of plasma cortisol in untreated sheep. In humans with EAS plasma cortisol levels are generally two to four fold higher than maximal, normal diurnal levels [11]. As shown in Fig. 1, ACTH infusion for 1 H or 24 H had no effect on renal 11βHSD2 mRNA levels ($P>0.05$). Therefore neither ACTH nor ACTH-dependent steroids appear to modulate the 11βHSD2 gene in the kidney. Activity levels of 11βHSD2 were also unaffected by ACTH treatment indicating that the level of renal enzyme present remains unaltered. Control: 3.8+/−0.2, 1 h ACTH: 3.8+/−0.6, 24 h ACTH: 3.2+/−0.4 ($P>0.05$). Results given as mean +/− standard error, percent conversion of cortisol per μg of protein.

It is therefore unlikely that ACTH is having a direct effect on renal 11βHSD2. Diederich *et al.*, (1996) suggest that the inhibition of 11βHSD2 activity in EAS is due to the elevated levels of glucocorticoids potentially overloading the enzyme [18]. The primary factor resulting in renal 11βHSD2 inhibition in EAS may be the elevated level of corticosterone present. In all species 11βHSD2 has a ten fold higher affinity for corticosterone compared to cortisol and therefore the elevated circulating levels of corticosterone in EAS ($10^{-8}-2\times10^{-7}$ M) may contribute significantly to the inhibition of renal 11βHSD2. 11βHSD2 activity is also under the regulation of end product inhibition and therefore elevated cortisone and 11-dehydrocorticosterone levels may also play a role in the inhibition of 11βHSD2 activity in EAS.

Studies by Li *et al.* (1996), in the rat, demonstrated the down regulation of renal 11βHSD2 gene expression, after treatment with adrenocorticoids (glucocorticoids and mineralocorticoids) [19]. In contrast we did not observe an effect of ACTH-dependent steroids on renal 11βHSD2 mRNA levels or activity in the sheep. A conundrum of the study in rats was the observation of increased enzyme activity levels parallel to decreased mRNA levels [19].

Up to 15% of untreated essential hypertension patients demonstrate renal 11βHSD2 inhibition. Whether this decrease is a primary or secondary cause is unclear. It is therefore important to determine factors inhibiting renal 11βHSD2 at the protein and gene level. Given that 11βHSD2 is clearly required for normal homeostasis, factors that alter its activity either at the gene level or the enzyme level are of potential (patho) physiological significance. To date little is known about the regulation of 11βHSD2

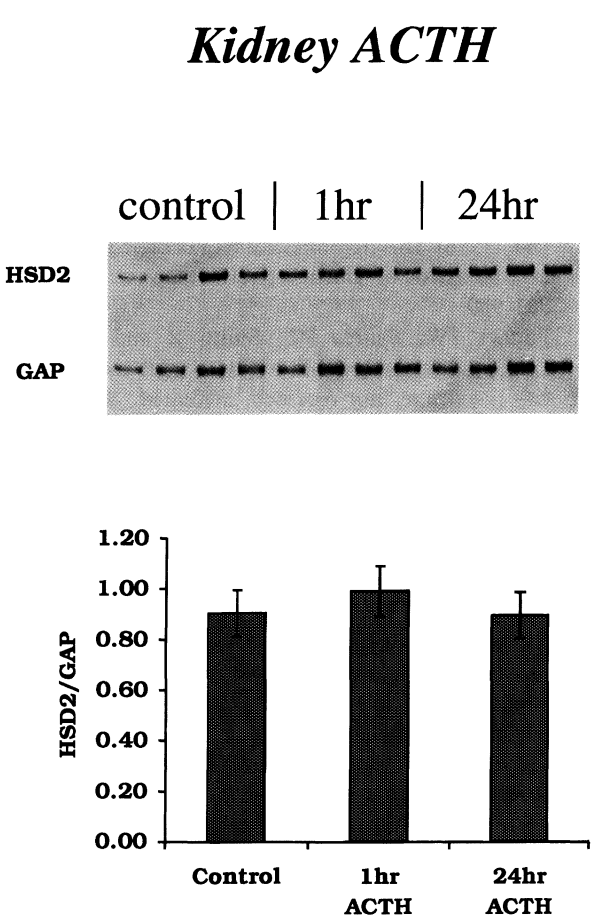

Fig. 1. Effect of ACTH on renal 11βHSD2 mRNA levels (a) Representative autoradiograph; exposure time 16 h. (b) Quantitation of mRNA levels was determined using a Fuji BAS 2000 Bioimaging Analyser. 11βHSD2 mRNA levels were normalized to GAPDH levels and expressed as mean +/−sem.

at the gene level. Pasquarette *et al.* [20] have shown an increase in mRNA levels in the human placental cell line, JEG-3, mediated by cAMP. In contrast we have observed a marked decrease in sheep, adrenal 11βHSD2 mRNA levels in the adrenal gland after ACTH treatment (Albiston & McDougall, unpublished observation).

We have demonstrated in this study that the expression of 11βHSD2 in the kidney is unaltered by ACTH. The reduced inactivation of cortisol by 11βHSD2 observed in EAS is likely to be in part due to end product inhibition or substrate overload of the enzyme by endogenous substrates (cortisol, corticosterone), rather than inhibition of 11βHSD2 at the transcriptional level by either ACTH or ACTH regulated steroids [18].

References

1. Krozowski ZS and Funder JW (1983) Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Proc Natn Acad Sci* USA 80: 6056–6060.
2. Arriza JL, Simerly RB, Swanson LW and Evans RM (1988) The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. *Neuron* 1: 887–900.
3. Funder JW, Pearce PT, Smith R and Smith AI (1988) Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 242: 583–585.
4. Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, deKloet ER and Monder C (1988) Localisation of 11 beta-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. *Lancet* 2: 986–989.
5. Stewart PM, Wallace AM, Valentino R, Burt D, Shackleton CH and Edwards CRW (1987) Mineralocorticoid activity of liquorice, 11 beta-hydroxysteroid dehydrogenase comes of age. *Lancet* 2: 821–824.
6. Ulick S, Levine SL, Gunczler P, Zanconato G, Ramirez LC, Rauh W, Rosler A, Bradlow LH and New MI (1979) A syndrome of apparent mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol. *J Clin Endocrinol Metab* 49: 757–764.
7. Mune T, Rogerson FM, Nikkila H, Agarwal AK and White PC (1995) Human hypertension caused by mutations in kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nature Genetics* 10: 291–399.
8. Wilson RC, Krozowski ZS, Li K, Obeyesekere V, Razzaghy-Azar M, Harbison MD, Wei JQ, Shackleton CH, Funder JW and New MI (1995) A mutation in the HSD11B2 gene in a family with Apparent Mineralocorticoid Excess. *J Clin Endocrinol Metab* 80: 2263–2266.
9. Soro A, Ingram MC, Tonolo G, Glorioso N and Fraser R (1995) Evidence for coexisting changes in 11 beta-hydroxysteroid dehydrogenase and 5 beta-reductase activity in subjects with untreated essential hypertension. *Hypertension* 25: 67–70.
10. Walker BR, Campbell JC, Fraser R, Stewart PM and Edwards CRW (1992) Mineralocorticoid excess and inhibition of 11 beta-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. *Clin Endocrinol* 37: 483–492.
11. Howlett TA, Drury PL, Perry L, Doniach I, Rees LH and Besser GM (1986) Diagnosis and management of ACTH dependent Cushing's syndrome: comparison of the features in ectopic and pituitary ACTH production. *Clin Endocrinol* Jun: 24: 699–713.
12. Eisenschmid B, Heilmann P, Oelkers W, Rejaibi R and Schonshofer M (1987) 20-Dihydroismers of cortisol and cortisone in human urine: excretion rates under differant physiological conditions. *J Clin Chem Clin Biochem* 25: 345–349.
13. Yang K and Yu M (1994) Evidence for distinct isoforms of 11 beta-hydroxysteroid dehydrogenase in the ovine liver and kidney. *J Steroid Biochem* 49: 245–250.
14. Albiston AL, Obeyesekere VR, Smith RE and Krozowski ZS (1994) Cloning and tissue distribution of the human 11 beta-hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* 105: R11–R17.
15. Chirgwin JM, Pryzbyla AE, MacDonald RJ and Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18: 5294–5299.
16. Butkus A, Albiston A, Alcorn D, Giles M, McCausland J, Moritz K, Zhuo J and Wintour E (1997) Ontogeny of angiotensin II receptors, types 1 and 2, in ovine mesonephros and metanephros. *Kidney Int* 51: 628–636.
17. Agarwal AK, Mune T, Monder C and White PC (1994) NAD(+)-dependent isoform of 11 beta-hydroxysteroid dehydrogenase-cloning and characterization of cDNA from sheep kidney. *J Biol Chem* 269: 25, 959–25962.
18. Diederich S, Quinkler M, Miller K, Heilmann P, Schoneshofer M and Oelkers W (1996) Human kidney 11 beta-hydroxysteroid dehydrogenase: regulation by adrenocorticotropin? *Eur J Endocrinol* 134: 301–307.
19. Li KX, Smith RE, Ferrari P, Funder JW and Krozowski ZS (1996) Rat 11 beta-hydroxysteroid dehydrogenase type 2 enzyme is expressed at low levels in the placenta and is modulated by adrenal steroids in the kidney. *Mol Cell Endocrinol* 120: 67–75.
20. Pasquarette MM, Stewart PM, Ricketts ML, Imaishi K and Mason JI (1996) Regulation of 11 beta-hydroxysteroid dehydrogenase type 2 activity and mRNA in human choriocarcinoma cells. *J Mol Endocrinol* 16: 269–275.