LOCALISATION OF 11β-HYDROXYSTEROID DEHYDROGENASE—TISSUE SPECIFIC PROTECTOR OF THE MINERALOCORTICOID RECEPTOR

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Summary In vitro the mineralocorticoid receptor is non-specific and does not distinguish between aldosterone and cortisol. In vivo certain tissues with this receptor are aldosterone selective (eg, kidney and parotid) whereas others with the same receptor are not (eg, hippocampus and heart). Experiments in rats showed that 11β-hydroxysteroid dehydrogenase (which converts cortisol to cortisone in man and corticosterone to 11-dehydrocorticosterone in the rat) was much more highly concentrated in aldosterone-selective tissues than in non-selective tissues. The localisation in the selective tissues was such that the enzyme could act as a paracrine or possibly an autocrine mechanism protecting the receptor from exposure to corticosterone. Autoradiographic studies showed that protection is lost when the enzyme is inhibited; ^{3}H-corticosterone and ^{3}H-aldosterone were bound to similar sites. These findings seem to explain why sodium retention, hypokalaemia, and hypertension develop in subjects with congenital deficiency of 11β-OHSD and those in whom the enzyme has been inhibited by liquorice.

Introduction

IN-VITRO experiments with the mineralocorticoid (type 1) receptor, either cytosolic preparations or cloned receptor expressed in transfected cells, have shown that its affinity is similar for aldosterone, cortisol, corticosterone, and deoxycorticosterone.[1-3] In vivo, by contrast, these type 1 receptors in the kidney, parotid, and colon are aldosterone-selective, though those in the hippocampus do not distinguish between aldosterone and corticosterone.[1,2] These results led Funder[4] to suggest that there must be a factor other than the receptor responsible for determining the aldosterone tissue specificity. He suggested that this might be extravascular corticosteroid binding globulin (CBG) which preferentially bound cortisol or corticosterone

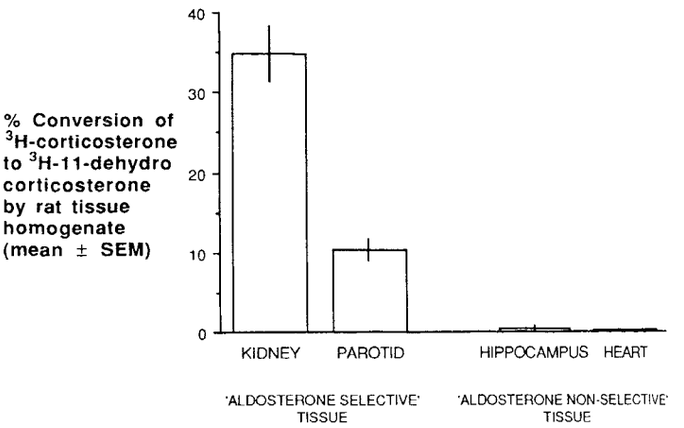

Fig 1—11β-OHSD activity in homogenates of rat renal cortex, parotid, hippocampus, and heart (n = 5).

(the major glucocorticoid in the rat). However, in the 10-day-old rat, which has very low levels of CBG, the in-vivo specificity of aldosterone was maintained despite the much higher levels of corticosterone.[5]

11β-hydroxysteroid dehydrogenase (11β-OHSD) is the microsomal enzyme complex responsible for the interconversion of cortisol and cortisone. It consists of two separate enzymes, one converting cortisol to cortisone (11β-dehydrogenase) and the other cortisone to cortisol (11-oxo-reductase).[6] Congenital deficiency of 11β-OHSD, originally described by Ulick,[7] is associated with severe hypertension, hypokalaemia, and suppression of plasma aldosterone and plasma renin activity—the syndrome of apparent mineralocorticoid excess. Our studies in an adult with this syndrome suggested that cortisol was acting as a mineralocorticoid.[8,9] The findings were in keeping with other results in Ulick's index case.[10] We hypothesised that the normal kidney used 11β-OHSD to convert cortisol to the inactive steroid cortisone and was thus protected from this effect. If this was so, then inhibition of 11β-OHSD would cause this protective mechanism to fail and allow access of cortisol to the non-specific renal mineralocorticoid receptors, resulting in sodium retention. We then found that the active component of liquorice (glycyrrhetinic acid) was a potent inhibitor of 11β-OHSD and proposed that this was the explanation for the sodium retaining and potassium losing actions of liquorice.[11] This answered the question why liquorice did not have these effects in severe adrenocortical insufficiency or after bilateral adrenalectomy.[11]

11β-OHSD is present in liver, kidney, gonads, placenta, lung, and intestinal mucosa.[12] The purification of 11β-OHSD has allowed the production of a specific antiserum

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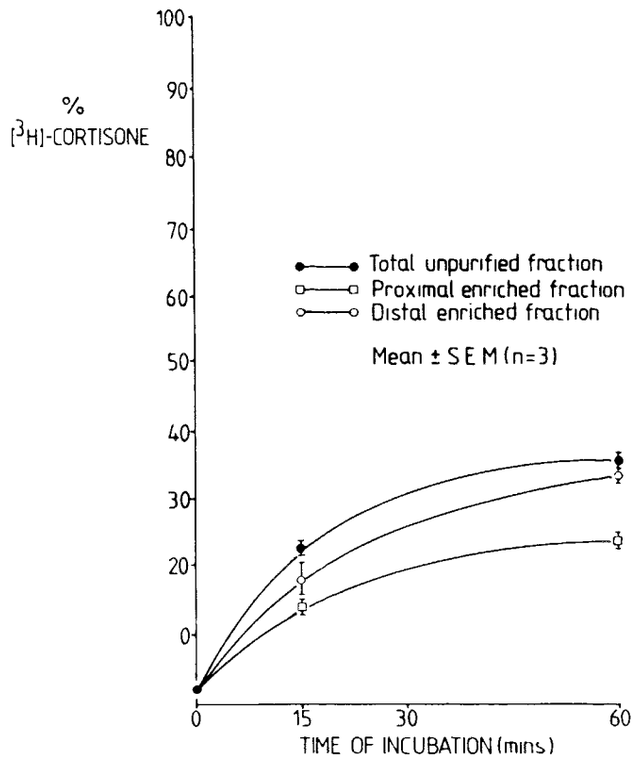

Fig 2—Time course of conversion of ^{3}H-cortisol to ^{3}H-cortisone by rat kidney cortical tubules.

and hence precise tissue localisation. We have now examined the tissue distribution of 11β-OHSD to determine whether the enzyme is appropriately situated to act as a paracrine or autocrine protector of the mineralocorticoid receptor in aldosterone-selective organs. In the usual paracrine system a hormone produced by one cell type acts on adjacent cells, whereas in an autocrine system the hormone is produced by and acts on the cell of origin. In this paper the term paracrine is used to denote the metabolism of the steroid by 11β-OHSD in cells which do not contain the mineralocorticoid receptor but which can influence the hormonal environment of other cells with the receptor. This contrasts with an autocrine system in which the enzyme and the receptor are in the same cells. In addition we have looked at tissues which are not aldosterone-selective but which have mineralocorticoid receptors, to determine whether this non-selectivity could be explained by an absence of 11β-OHSD. Finally we have examined the effect of 11β-OHSD inhibition on the specific binding of corticosterone by the kidney, to determine whether this would result in corticosterone binding in the same sites as aldosterone.

Methods

Measurement of Enzyme Activity

Renal cortex, parotid, heart, and hippocampus were obtained from 10-day-old male Sprague-Dawley rats and 0·5 g wet weight tissue was homogenised in 10 ml Krebs-Ringer buffer with a Dounce tissue grinder for 30 strokes. Using a fixed mg protein/g wet weight tissue (Bio-Rad protein assay kit) we incubated 400 μl of a diluted homogenate preparation at 37°C for 60 min with 600 μl Krebs-Ringer buffer (+0·2% glucose, 0·2% bovine serum albumin) and $1{\cdot}2 \times 10^{-8}$ mol ^{3}H-corticosterone (specific activity 84 Ci/mmol, Amersham International). After centrifugation, steroids were extracted from the supernatant with ethyl acetate and ^{3}H-corticosterone was separated from ^{3}H-11-dehydrocorticosterone by thin-layer chromatography. The percentage conversion of corticosterone to 11-dehydrocorticosterone by 11β-OHSD was then calculated.

Isolation of Rat Kidney Cortical Tubules

2 g wet weight of renal cortical tissue was taken from 3-month-old male Sprague Dawley rats and tubules were prepared by use of a mechanically dispersed enzyme solution (0·05% w/v collagenase, 0·1% w/v hyaluronidase).[13] After filtration (to remove glomeruli) and successive washing procedures the tubules were subjected to unit gravity sedimentation through a Ficoll gradient.[14] From this both a distal enriched fraction (narrow, transparent tubules) and a proximal enriched fraction (broad, yellowish tubules) were established with purities of 85% and 70%, respectively. Tubular integrity was evaluated by the dye exclusion method.[15] A tubular count was made with a haemocytometer and 400 μl of each tubular preparation (containing about 10^5 tubules) was incubated with 600 μl Krebs-Ringer buffer (containing 0·2% glucose, 0·2% bovine scrum albumin) and $1{\cdot}2 \times 10^{-8}$ mol ^{3}H-cortisol. Steroids were extracted and separated as above and 11β-OHSD activity was calculated in proximal and distal tubules as percentage conversion of cortisol to cortisone.

Localisation of 11β-OHSD Activity

11β-dehydrogenase has been purified to apparent homogeneity[16] from a rat hepatic microsomal preparation. 800-fold purification was achieved by agarose-NADP affinity chromatography. The purified enzyme is a glycoprotein (molecular weight 34 000) and has no reductase activity. Antibodies to this homogeneous 11β-dehydrogenase were raised in female New Zealand white rabbits. Preimmune and immune sera were stored as 1 ml samples at −20°C. 3-month-old male Sprague Dawley rats (Charles River, Kent) were killed and the kidneys, parotid, heart, and hippocampus were removed, sliced longitudinally, and placed in Bouin's fixative for 24 h. They were then processed through to paraffin blocks and sections were cut at 4 μm. Immunostaining was done by the avidin-biotin-peroxidase method[17] with Vector Elite reagents (Vector Laboratories, Burlinghame, California, USA).

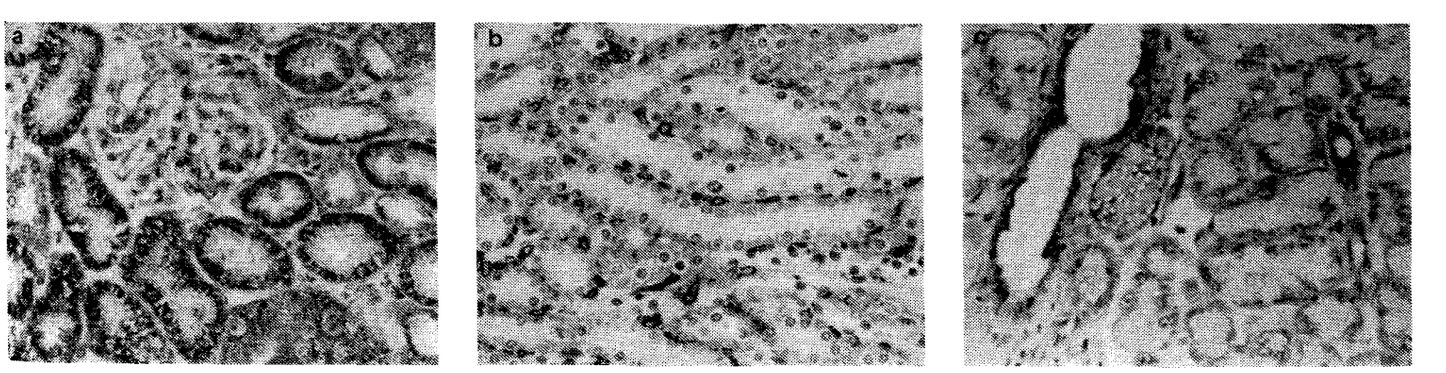

Fig 3—Immunohistochemical localisation of 11β-OHSD.

(a) Concentration in proximal rather than adjacent distal tubule; (b) concentration in intertubular space and vasa recta, not medullary collecting tubule; (c) concentration in parotid intercalated and striated ducts but not in acini. All × 25, reduced by about half.

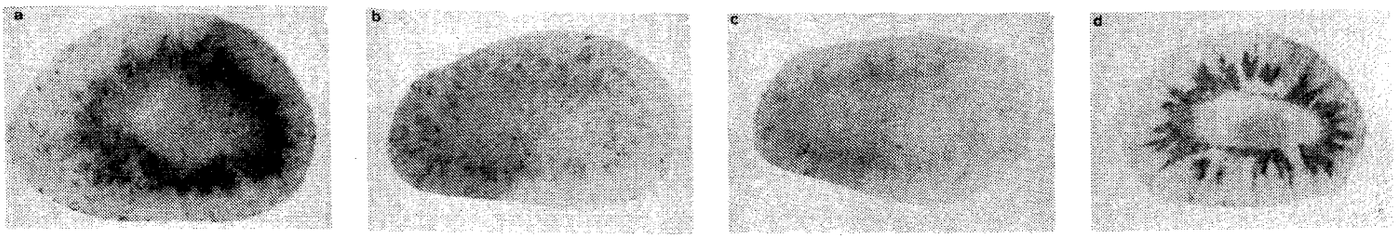

Fig 4—Renal autoradiographs.

(a) After ^{3}H-aldosterone; (b) after ^{3}H-corticosterone; (c) after unlabelled corticosterone followed by ^{3}H-corticosterone; (d) after glycyrrhizic acid followed by ^{3}H-corticosterone. Method of Sutanto et al.[30]

Effects of 11β-OHSD Inhibition on Localisation of ^{3}H-corticosterone in Kidney

^{3}H-aldosterone (100 μCi/100 g body weight) or ^{3}H-corticosterone (100 μCi/100 g body weight) was given to adult Wistar rats via a jugular vein cannula 1 h before the animals were killed. In a separate experiment 5 mg glycyrrhizic acid was given subcutaneously 60 min before the ^{3}H-corticosterone to inhibit 11β-OHSD. In a further experiment 1 mg unlabelled corticosterone was given subcutaneously 30 min before the dose of ^{3}H-corticosterone to determine non-specific binding. After death kidneys were removed, frozen, cryostat sectioned (25 μm thickness), and exposed to ^{3}H-'Ultrofilm' for 2 weeks.

Results

Fig 1 shows the activity of 11β-OHSD in the homogenates of renal cortex, parotid, hippocampus, and heart. The highest activity was present in the kidneys with lower levels in the parotid. Little or no enzyme was present in the hippocampus or heart.

Density gradient separation showed that both the proximal and the distal tubular preparations were capable of converting cortisol to cortisone (fig 2). In the three experiments perfomed, enzyme activity was higher in the distal than in the proximal tubule. The high level of enzyme present within the kidney was confirmed by immunohistochemistry. In contrast to the findings with density gradient separation, the enzyme was mainly in the proximal tubule and not in the distal nephron (fig 3, a). However, the enzyme seemed also to be localised either in or immediately adjacent to the vasa recta alongside the papillary collecting tubules (fig 3, b). In the parotid 11β-OHSD was present in both the intercalated and the striated ducts but was not found in the acini (fig 3, c). No localised enzyme was present in the heart or hippocampus.

Autoradiography with ^{3}H-aldosterone showed the expected binding in the cortex/outer medulla and papilla/inner medulla (fig 4, a). In contrast, the uptake of ^{3}H-corticosterone in these sites was very low (fig 4, b) and little different from the non-specific binding (fig 4, c). However, after inhibition of 11β-OHSD the pattern of ^{3}H-corticosterone binding was greatly changed and was now similar to that with ^{3}H-aldosterone (fig 4, d).

Discussion

These investigations have shown that two major aldosterone-selective tissues (kidney and parotid) have much higher levels of 11β-OHSD than those organs with the same mineralocorticoid receptor but which are not aldosterone specific (heart and hippocampus). The position of enzyme in the kidney as assessed by immunohistochemistry (proximal tubule and vasa recta) suggests that the enzyme is well placed to act as a paracrine protector of the type 1 receptor in the cortical and papillary collecting tubule. The localisation of this receptor in the rat has been demonstrated by Shepherd and Funder[5] using tritiated aldosterone binding; they found it in both renal cortex/outer medulla and papilla/inner medulla—results in keeping with those obtained by Farman and Bonvalet who measured aldosterone binding along the nephron and showed high levels of nuclear labelling in the cortical collecting tubule.[18] The density gradient studies confirmed the presence of enzyme activity in the proximal tubule but also suggested that there was conversion of cortisol to cortisone by the distal nephron. One possible explanation for these apparently discrepant results is that there may be a different 11β-dehydrogenase in the distal tubule. Monder and Shackleton[12] have reviewed the kinetic evidence for multiple 11-dehydrogenases.

The free (non-protein-bound) fraction of cortisol in plasma is filtered by the kidney: 80–90% is reabsorbed passively by the tubule and only about 0·5% is excreted unchanged in the urine.[19,20] If this reabsorption takes place in the proximal tubule then the cortisol could be metabolised to cortisone. Any cortisol that was not filtered might be dealt with after diffusion from the peritubular capillaries into the proximal tubular cell. Hellman and colleagues[21] suggested that 11β-OHSD was involved in the cellular capture mechanism for cortisol and that, at least in the kidney, oxidation to cortisone was a prerequisite for cellular release of the steroid. By contrast aldosterone, which is metabolised very little by 11β-OHSD, would be reabsorbed and pass into the peritubular plexus without inactivation. Aldosterone and the inactive glucocorticoid metabolite would then pass down the peritubular plexus to reach the collecting tubule where aldosterone diffuses across the basolateral cell membrane to gain access to the cytoplasmic or possibly intranuclear receptor.

The blood supply to the renal medulla is such that 11β-OHSD would need to be sited elsewhere to prevent access of cortisol to the papillary collecting tubule. Some vasa recta arise directly from interlobular arteries and do not have an initial circulation through a glomerular tuft. In this case the enzyme would need to be in close relation to the descending vasa recta. Our immunohistochemistry results are in keeping with this notion.

A crucial test of our paracrine hypothesis is the demonstration that inhibition of 11β-OHSD results in loss of the selectivity of the binding of aldosterone in tissues such as the kidney. The autoradiography results strongly suggest that this is so. When the enzyme is intact, corticosterone (the steroid equivalent to cortisol in man) is not taken up by the kidney. However, when 11β-OHSD is inhibited by glycyrrhizic acid (an ingredient of liquorice that is

hydrolysed in vivo to the major active component glycyrrhetinic acid) the aldosterone selectivity is lost and corticosterone now binds in a distribution similar to that of aldosterone. It remains to be determined whether all the corticosterone uptake is by the same receptors as those that bind aldosterone. Using isolated tubules Kurt Lee and colleagues[22] showed that corticosterone binding sites were concentrated in the cortical collecting tubule. Part but not all of this steroid could be displaced by unlabelled aldosterone. In these experiments corticosterone had direct access to the nephron from the incubation medium and was thus able to bypass the protective moat provided by 11β-OHSD. The results thus favour a paracrine rather than an autocrine system.

In the parotid the enzyme was found in both the intercalated ducts and the striated ducts but not in the acini. Previous studies with micropuncture have shown that sodium reabsorption and potassium secretion occurs in the striated but not in the intercalated ducts in rat submaxillary glands[23]—findings in keeping with the salivary gland localisation of $[Na^+ + K^+]$-ATPase in the cat as assessed by means of ^{3}H-ouabain, which was heavily concentrated in the cells of the striated duct.[24] These results suggest that the type 1 receptor is likely to be in the striated duct.

How might mineralocorticoids gain access to the striated duct? The blood supply to the parotid is very different from that in the kidney. The arteries accompany the ducts within the lobules and break up into capillary and precapillary plexuses around the striated ducts. Arteriolar arcades then arise which continue to supply the acini. Blood flow data suggest that the flow is mainly countercurrent to that of saliva.[25] If aldosterone then entered via the basal cell membrane of the striated duct then 11β-OHSD would need to be present within the cells of the duct. This seems to be what happens, and is in keeping with an autocrine system. If, however, aldosterone enters via the luminal membrane then this would allow the possibility of upstream metabolism by the enzyme in the intercalated duct.

The very low levels of the enzyme in the hippocampus and the heart associated with the lack of any specific tissue localisation suggest that these tissues contain no 11β-hydroxysteroid dehydrogenase mechanism for the inactivation of cortisol or corticosterone. These steroids would thus have direct access to the receptor—an interpretation consistent with previous work showing that there are type 1 receptors in these tissues but they are not aldosterone-selective.[1-3,5,26] In fact, behavioural and biochemical studies in the rat have revealed several responses that are under stringent control of corticosterone acting via the limbic type 1 receptor. Aldosterone seemed to be a competitive antagonist.[27,28]

Human 11β-OHSD has yet to be purified and so no antisera have been produced, but measurement of enzyme activity leads us to believe that tissue localisation would be similar to that we have found in the rat. The human kidney is an important site for the conversion of cortisol to cortisone,[21] and in patients with renal disease we would expect this mechanism to be impaired. We have lately shown that plasma cortisone levels are reduced in such patients and there is a strong negative correlation between plasma cortisone and creatinine.[29] It remains to be determined what part this might play in the sodium retention of renal failure. The lack of renal 11β-OHSD might also be important in delaying the development of hyperkalaemia in such patients. These effects would require the loss of enzyme activity to be dissociated from that of the mineralocorticoid receptors in the collecting tubule.

These findings all point to the importance of this steroid shuttle. Congenital or acquired deficiency of the enzyme converting cortisol to cortisone means that cortisol functions as a potent mineralocorticoid. We suggest that without this paracrine mechanism we would be "pillars of salt".

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