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THE CORTISOL–CORTISONE SHUTTLE AND THE APPARENT SPECIFICITY OF GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS

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Summary—*In vitro* studies on both the purified cytosolic mineralocorticoid receptor (MR) and the recombinant expressed human MR have shown that it is non-specific and does not distinguish between cortisol and aldosterone. These contrast with the apparent *in vivo* selectivity of the MR in tissues such as the kidney for aldosterone in preference to cortisol despite the 100-fold molar excess of cortisol. This review gives the evidence that indicates that 11β-hydroxysteroid dehydrogenase (11β-OHSD), the enzyme responsible for the interconversion of cortisol and inactive cortisone, acts as a protective mechanism for the MR. In aldosterone-selective tissues it shuttles cortisol to cortisone and thus prevents glucocorticoid access. Aldosterone itself is not a substrate for the enzyme. The current data suggest that this is an autocrine system with both the enzyme and the MR present within the same cell. In certain tissues such as the kidney there may also be additional upstream steroid metabolism indicating a paracrine system. Lack of this protective system results in cortisol acting as a potent mineralocorticoid. This may be congenital as in the apparent mineralocorticoid excess syndrome or acquired secondary to liquorice-induced inhibition of 11β-OHSD. In addition to its role in MR protection 11β-OHSD may also be important in modulating steroid access to glucocorticoid receptors. The ontogeny of the enzyme in the testis and the brain suggests that its tissue-specific control may be crucial in allowing normal development.

INTRODUCTION

The role of defects of the cortisol–cortisone shuttle in the genesis of hypertension has been discussed in the clinical part of this review which covered the apparent mineralocorticoid excess syndrome (AME) and the mineralocorticoid excess secondary to liquorice ingestion [1]. The former results from congenital deficiency of 11β-hydroxysteroid dehydrogenase (11β-OHSD) and usually presents with severe hypertension and hypokalaemia in childhood, and the latter is secondary to inhibition of 11β-OHSD by glycyrrhetinic acid (GE), the active component of liquorice. The second part will concentrate on the mechanism responsible for the sodium retention and hypertension, and will review the evidence that indicates that 11β-OHSD acts as a tissue-specific protector for the mineralocorticoid receptor (MR) and can modulate access of glucocorticoids to their own receptor (GR).

The original studies on the binding of adrenal steroids suggested that there were two types of high affinity binding site [2]. One of these, present in rat kidney extracts, had a high affinity for aldosterone and a lower affinity for the synthetic glucocorticoid dexamethasone and was termed the Type I or MR. The other site had a high affinity for glucocorticoids and was called the Type II receptor [3].

Further studies showed that the Type I receptor was found not only in tissues involved with the transport of sodium across epithelia (kidney [4–6], parotid [4, 7] and colon [8, 9]) but also in the hippocampus [10–12] and heart [13]. *In vivo* studies on the binding of glucocorticoids in these tissues showed marked differences. The kidney, parotid and colon were "aldosterone-selective" and bound aldosterone in preference to corticosterone (B), whereas the hippocampus and heart were "non-selective" and did not distinguish aldosterone and cortisol [14, 15].

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The cloning of the human MR (hMR) by Arriza *et al.* [16] allowed transfection studies to be carried out. These showed that the hMR was apparently non-selective and had an equal affinity for aldosterone, B and cortisol. As the circulating levels of free cortisol are 100 times those of aldosterone this indicated that there must be some mechanism other than the receptor structure which was responsible for its apparent specificity *in vivo*. Funder *et al.* [17] suggested that this might be cortisol binding globulin (CBG) which has a much higher affinity for cortisol than aldosterone. However, studies in 10-day-old rats which are CBG deficient showed that the *in vivo* specificity of the MR was maintained [14]. Thus CBG could not be the answer.

11β-OHSD AND THE MR

The clinical part of this review detailed the evidence that cortisol acts as a potent mineralocorticoid in both congenital and acquired deficiency of 11β-OHSD [1]. This suggested that in these conditions the normal specificity of the hMR is lost and led us to propose that 11β-OHSD is normally responsible for protecting the MR from exposure to cortisol in aldosterone-selective tissues such as the kidney [18, 19]. In these circumstances cortisol would not be "seen" by the receptor because of metabolism to inactive cortisone. In contrast aldosterone (which is not a substrate for 11β-OHSD, presumably because of its 11,18 bridge structure) would have direct access to the MR.

To test this hypothesis we measured conversion of corticosterone (B) (the equivalent of cortisol in the rat) to 11-dehydrocorticosterone (A) by rat tissue homogenates with a fixed mg protein/g wet weight tissue [18]. In "aldosterone non-selective" tissues such as the heart there was very low 11β-OHSD activity as judged by conversion of B to A. By contrast high levels of activity were present in "aldosterone-selective" tissues (kidney and parotid). We then performed autoradiographic studies to look at the binding and localization of tritiated B in rat kidney. After bilateral adrenalectomy Wistar rats were given [^{3}H]B via a jugular vein cannula. The tracer dose used was one which had been previously shown to saturate the non-selective Type I MR in the hippocampus but was insufficient to produce a signal from the lower affinity Type II receptor. One hour later the animals were killed. In a separate experiment 5 mg glycyrrhizic acid (which is converted to GE *in vivo*) was given s.c. 60 min before the [^{3}H]B to inhibit 11β-OHSD. In a further experiment 1 mg unlabelled B was given s.c. 30 min before the dose of [^{3}H]B to determine non-specific binding. In a final experiment 1 mg aldosterone was administered to an animal given glycyrrhizic acid and [^{3}H]B to determine whether it was possible to displace the labelled steroid from Type I receptors. The kidneys were removed, cryostat sectioned and exposed to ^{3}H-autofilm for 2 weeks. The results are shown in Fig. 1. In the animal given [^{3}H]B alone no significant uptake was found and there was no difference between the autoradiograph and that from the animal given unlabelled B prior to [^{3}H]B. When however [^{3}H]B was given after inhibition of 11β-OHSD by glycyrrhizic acid a marked difference was observed with uptake of the label in sites known to possess

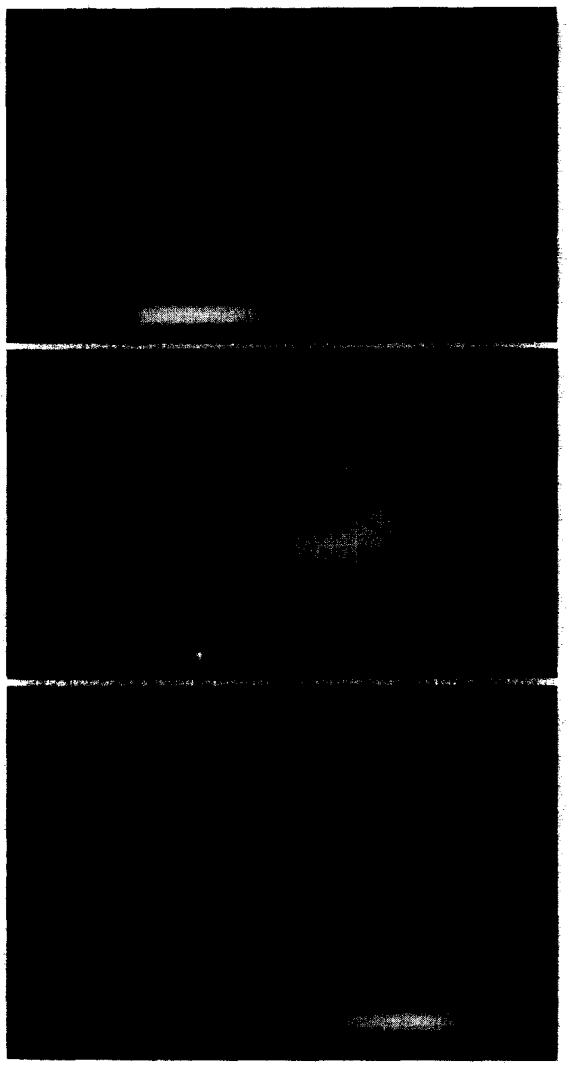

Fig. 1. Autoradiographs of kidneys from rats given: (a) [^{3}H]B; (b) glycyrrhizic acid followed by [^{3}H]B; and (c) combination as in (b) followed by unlabelled aldosterone.

the MR, i.e. cortex outer medulla and papilla inner medulla. This suggested that inhibition of 11β-OHSD was allowing access of the [^{3}H]B to the MR. To confirm this the experiment with unlabelled aldosterone was performed. This showed that it was possible to displace the [^{3}H]B and indicated that the glucocorticoid had been bound to the MR.

At our suggestion John Funder then further tested the protective role of 11β-OHSD by repeating his *in vivo* binding studies with [^{3}H]aldosterone and [^{3}H]B given to adrenalectomized rats with and without inhibition of 11β-OHSD by carbenoxolone [21]. He then homogenized the tissues and after ultracentrifugation added hydroxylapatite to the cytosol preparations to separate receptor-bound from free steroid. In the hippocampus and heart the Type I receptor was non-selective and showed no difference in binding of [^{3}H]aldosterone and [^{3}H]B in the presence or absence of carbenoxolone. By way of contrast the kidney, parotid and colon showed selective aldosterone binding. This selectivity was however lost when carbenoxolone was given suggesting that this had removed the protective mechanism.

To look further at this relationship between the MR and 11β-OHSD we have collaborated with Bernard Rossier in Lausanne using the urinary bladder of *Bufo marinus* [22]. Specific binding sites for glucocorticoids have been demonstrated in the toad bladder with a high affinity, low capacity Type I receptor and a low affinity, high capacity Type II receptor [23, 24]. As with the rat the purified cytosolic Type I receptor shows similar affinities for aldosterone and B. Previous work had shown that the bladder had 11β-OHSD activity and rapidly converted B to A. Using immunohistochemistry with an antibody raised against the rat enzyme we showed that there was immunoreactivity in the bladder with the highest levels in the granular cells of the epithelial layer (Fig. 2). To test whether this could protect the Type I receptor in these cells we measured sodium transport across the bladder as assessed by short circuit current and electrical resistance. When low dose aldosterone (10 nM) was applied to the serosal side it produced an increase in sodium transport whereas B (10 nM) was ineffective. Carbenoxolone alone did not alter sodium transport. However, when carbenoxolone was given and then 2 h later B the effect on sodium was marked and not significantly different from that produced by aldosterone (Fig. 3). Similar studies have been carried out by David Morris's group who have also shown the very rapid metabolism of B to A by *in vitro* bladder incubations [25].

Fig. 2. Immunohistochemistry showing localization of 11β-OHSD in toad bladder. For details of methods see Ref. [18].

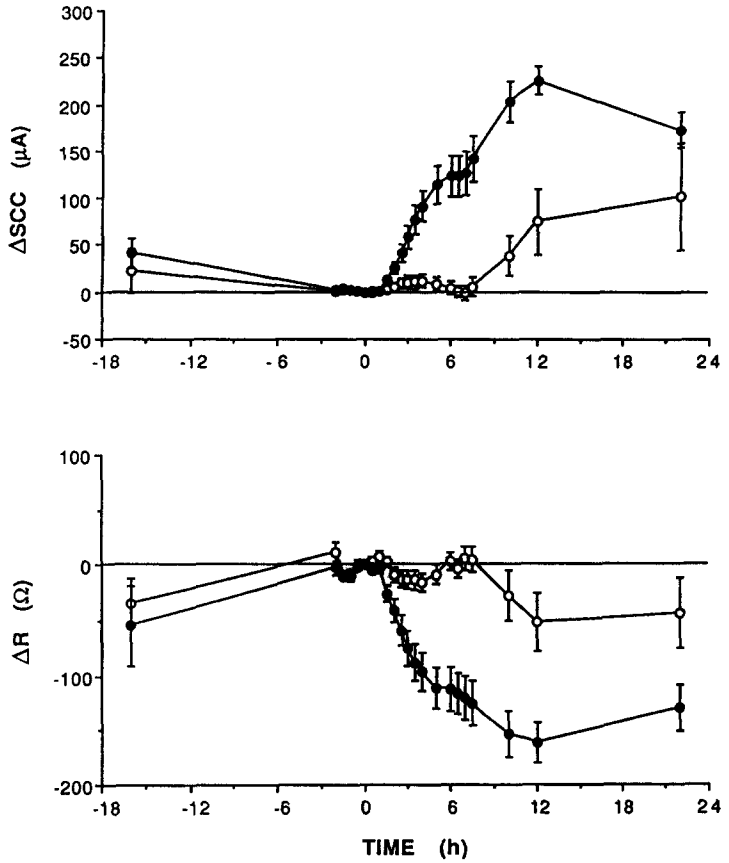

Fig. 3. Effects of carbenoxolone on the response (mean ± SEM) of short circuit current and electrical resistance across toad bladder in response to B. Carbenoxolone (10 μM serosal side) added at −2 h to test hemibladders (●) and diluent to control hemibladders (○). B (10 mM) was added to serosal side only at time 0. At 6 h B (10 nM) was added to the mucosal side of both test and control hemibladders. For details see Ref. [22].

Interestingly in our experiments this protective effect of 11β-OHSD was only manifest when the B was applied to the serosal side. On the mucosal side B and aldosterone were equally effective. This might indicate that the 11β-OHSD activity was greater in the submucosa and serosal tissue than in the target cell containing the MR. The immunohistochemistry would be against this. Alternatively there may be a specific subcellular localization of the enzyme at the basolateral membrane or in the endoplasmic reticulum near the basolateral membrane.

To examine the physiological relevance of 11β-OHSD Souness and Morris [26] tested the effect of carbenoxolone on the renal actions of B and cortisol in adrenalectomized Sprague–Dawley rats. The animals were given carbenoxolone 2.5 mg s.c. or vehicle and then 30 min later s.c. 3 ml 0.9% NaCl together with varying doses of B or cortisol or control vehicle. In the animals pretreated with vehicle none of the doses of B or cortisol had significant effects on urinary sodium excretion. However, in those pretreated with carbenoxolone the two higher doses of B (100 and 500 μg/rat) produced sodium retention as did the only dose of cortisol used (1 mg/rat).

DOES 11β-OHSD PROVIDE PARACRINE OR AUTOCRINE MR PROTECTION?

In our original paper we used two methods to determine the intra-renal localization of 11β-OHSD and hence its relationship to the MR-density gradient separation of proximal and distal tubular fractions with measurement of 11β-OHSD activity, and immunohistochemistry [18].

The density gradient studies indicated that there was 11β-OHSD in both proximal and distal tubular fractions. This might suggest both an upstream paracrine role for 11β-OHSD and an autocrine mechanism in the mineralocorticoid target cells. However, to our surprise the immunohistochemistry showed that the enzyme was mainly in the proximal tubule and

not in the distal nephron. The latter results were confirmed by Rundle *et al.* [27] using the same antiserum and an antibody against the MR. The recent elegant work by Fejes-Toth *et al.* [28] in the rabbit would suggest that the immunohistochemistry may be misleading. She examined whether metabolism of B occurs in the renal target cells of aldosterone, i.e. in the cortical collecting duct cells. These were isolated by solid phase immunoadsorption using an antiserum raised against the cells. She then looked at B metabolism in the freshly isolated cells and in primary cultures grown in monolayers. The former rapidly converted B to A (95% conversion after 30 min). When B was added to one side of the monolayer virtually all the steroid recovered from the contralateral side after 3 h of incubation was A (only $0.25 \pm 0.24\%$ B could be detected). Thus B could not traverse the cells without conversion. When, however, carbenoxolone was added (2×10^{-5} M) the conversion was markedly inhibited and $76.9 \pm 8.8\%$ of the labelled B was on the contralateral side. These results strongly support an autocrine system. However, they do not exclude an additional upstream paracrine role for 11β-OHSD.

We have recently used *in situ* hybridization to determine the renal localization of the mRNA for 11β-OHSD [29]. The results have shown that the mRNA is present in both proximal and distal tubules. The lack of immunoreactivity in the distal nephron thus raises the possibility of altered post-translational processing of 11β-OHSD in the distal nephron. This situation appears to be different in the central nervous system where both we and others have shown colocalization of 11β-OHSD and the Type I receptor [30, 31].

11β-OHSD AND THE GR

Having demonstrated the critical role of 11β-OHSD in relation to the MR we then looked at the possibility that it might modulate endogenous glucocorticoid access to the GR. Several pieces of evidence suggested this. In 1957 Kumagai *et al.* [32] looked at the corticoid-like action of glycyrrhizine and observed potentiation and prolongation of the effects of ACTH and hydrocortisone. Both glucocorticoid and mineralocorticoid actions were enhanced. The glucocorticoid effects were anti-inflammatory as judged by joint pain and pyrexia in a patient with acute rheumatic fever, pyrexia, sedimentation rate, rash and urine output in a patient with acute disseminated lupus erythematosus, and a reduction in eosinophil count in normal subjects. To look at this further we used the classic skin vasoconstrictor assay which has long been used to test the potency of topical glucocorticoids. We demonstrated that 11β-OHSD activity was present in the skin and using immunohistochemistry showed that the enzyme was in the epidermis [33]. When hydrocortisone alone was applied to the skin it had negligible vasoconstrictor effect. However, the addition of GE to inhibit 11β-OHSD produced marked potentiation. GE alone had no effect.

Further evidence, albeit indirect, comes from the demonstration of 11β-OHSD in tissues with high concentrations of GR but low levels of MR. Two good examples of this are the very high levels of 11β-OHSD bioactivity present in the cerebellum [34] and the interstitial cells of the testis [35]. In both these sites the ontogeny of the enzyme suggests that it may be important in local control mechanisms. In the cerebellum bioactivity is very high during late foetal development in the rat but falls rapidly during the first 5 days of post-natal life and then rises again towards adult values by the 10th day. The peaks of enzyme bioactivity correlate well with the times of maximal sensitivity of cerebellar neurons to glucocorticoid-induced degeneration. In the rat testis the enzyme is not present until about 26 days of age and then progressively increases to adult levels. The lack of protection of the interstitial cells from glucocorticoid exposure in the first 26 days may be important in inhibiting gonadotrophin-induced testosterone production.

In an attempt to look at the functional significance of brain 11β-OHSD we have looked at the effect of GE administration on [^{14}C]2-deoxyglucose uptake in unstressed conscious rats [36]. When GE was given there was significant uptake only in the arcuate nucleus and preoptic area. However, when exogenous B (0.5 mg) was given after GE there was additional uptake in the parietal cortex layer IV, hippocampus CA3, zona incerta and paraventricular nucleus. B alone had no effect on glucose use in any region. When the effects were related to 11β-OHSD activity it was clear that those brain regions with high enzyme activity were the ones that showed alterations when 11β-OHSD was inhibited. When high doses of B were required to produce change it would seem likely that this effect was via increased access of B to GR.

An alternative approach is to determine whether inhibition of 11β-OHSD has an effect

in patients with pseudohypoaldosteronism. The problem with this approach is the relative lack of specificity of the current inhibitors of 11β-OHSD. Funder *et al.* [37] looked at the effect of carbenoxolone in four members of a family with this condition. All were salt supplemented for 19 days and then given carbenoxolone (150–300 mg daily) from day 5–19. On carbenoxolone there was a rise in serum bicarbonate and a fall in urinary Na/K ratio. They interpreted the blunted mineralocorticoid changes in the absence of MRs as evidence for either the unveiling of an electrolyte effect of cortisol via GR in the proximal tubule, an effect normally forbidden by 11β-OHSD in these cells or the action of cortisol via distal tubular GR acting as an inducer of what is normally mineralocorticoid responsive gene expression. The difficulty with these explanations is that it, if either of them is correct, it should then be possible to reproduce the effects of carbenoxolone by a glucocorticoid which is not metabolized by 11β-OHSD such as dexamethasone.

Very interesting recent results have come from a study of colonic Na K-ATPase gene expression induced by adrenocortical steroids [38]. The authors showed that dexamethasone produced a rapid increase of mRNA for the alpha-subunit (6-fold) but that aldosterone in high physiological dose had no effect. Carbenoxolone produced a 3-fold increase in intact but not adrenalectomized rats. This again suggests the possibility that 11β-OHSD may have a specific and separate GR related role.

In his perspective on "Adrenal Steroids: New Answers, New Questions" [39] John Funder suggested that two of the big questions remaining in the area of adrenal steroid physiology were how aldosterone occupies Type I receptors in mineralocorticoid target tissues in the face of much higher glucocorticoid concentrations, and the implications of both high-affinity (Type I) and low affinity (Type II) receptors for glucocorticoids. It would seem likely that our studies starting with a very rare cause of hypertension associated with 11β-OHSD deficiency have produced the answer to the first of these questions. The demonstration of the existence of the protective role of 11β-OHSD in amphibia suggests that this mechanism has been conserved over 300 million years of evolution. Further understanding of this system promises to shed light on a wide variety of physiological and pathophysiological processes.

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