Inhibition of renal 11β-hydroxysteroid dehydrogenase in vivo by carbenoxolone in the rat and its relationship to sodium excretion

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ABSTRACT

1. The type 2 isoform of 11β-hydroxysteroid dehydrogenase, an enzyme which converts cortisol or corticosterone to inactive 11-ketosteroid metabolites, is thought to be responsible for preventing access of endogenous glucocorticoids to mineralocorticoid receptors in the distal nephron; although direct in vivo evidence for this is still lacking. We have examined whether graded inhibition of renal 11β-hydroxysteroid dehydrogenase activities in vivo results in corresponding changes in urinary electrolyte excretion due to exposure of mineralocorticoid receptors to circulating endogenous glucocorticoids.

2. Anaesthetized rats were infused intravenously with vehicle alone or with one of three doses of carbenoxolone: 0.06, 0.6 or 6 mg/h. After measurement of renal electrolyte excretion, the kidneys were snap-frozen in liquid nitrogen and 11β-hydroxysteroid dehydrogenase activities were measured directly by enzyme assay in the presence of NAD⁺ or NADP⁺.

3. A dose-dependent inhibition of renal 11β-hydroxysteroid dehydrogenase activities was observed: the low, intermediate and high doses of carbenoxolone causing approximately 50%, 80% and > 90% inhibition respectively. Only with the high dose was an effect on renal function observed (decreased fractional Na⁺ excretion and urinary Na⁺/K⁺ ratio).

4. The poor correlation between the extent of inhibition of renal 11β-hydroxysteroid dehydrogenase and altered urinary Na⁺ excretion, apparent at the lower doses of carbenoxolone, suggests either that 11β-hydroxysteroid dehydrogenase has considerable functional reserve, or that it may not be the only mechanism determining mineralocorticoid receptor specificity in the distal nephron.

INTRODUCTION

In vitro studies have shown that mineralocorticoid receptors (MRs) have equal affinity for aldosterone and glucocorticoids [1]. In vivo, however, despite circulating levels of glucocorticoids approximately 100-fold greater than those of aldosterone, the MRs of the distal nephron display specificity for aldosterone. It is generally believed that this specificity is conferred by the microsomal enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD; HSD, hydroxysteroid dehydrogenase; MR, mineralocorticoid receptor.

Key words: carbenoxolone, fractional sodium excretion, glucocorticoids, 11β-hydroxysteroid dehydrogenase.
Abbreviations: CBX, carbenoxolone; GFR, glomerular filtration rate; HSD, hydroxysteroid dehydrogenase; MR, mineralocorticoid receptor.
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β-tissues, including the distal nephron [2–4].

To date, two isoforms of 11β-HSD have been cloned. Type 1 11β-HSD is a ubiquitously expressed NADP-dependent isozyme which has a low affinity for substrate and appears to act predominantly as an 11-ketosteroid reductase in the liver, from which it was originally isolated [5,6]. In contrast, type 2 11β-HSD is an NAD+-dependent, high-affinity dehydrogenase enzyme, expressed specifically in mineralocorticoid-sensitive tissues, i.e. distal nephron, colon and parotid glands [7,8]. After the cloning of 11β-HSD2, it emerged that mutations in the gene encoding this isozyme are associated with the congenital form of the syndrome of ‘apparent mineralocorticoid excess’, a condition characterized by sodium retention and hypokalaemia despite normal or low levels of aldosterone [9,10].

Despite this clear evidence linking genetic defects of 11β-HSD2 with altered renal function, studies attempting to inhibit 11β-HSD pharmacologically have yielded less consistent observations regarding the biochemical and physiological roles of the enzyme. The agent commonly used is the hemisuccinate ester of glycyrrhetinic acid, carbenoxolone (CBX), which has been shown to inhibit 11β-HSD activities in vitro [2,11]. Although in a study in healthy humans the oral administration of CBX was found to decrease urinary sodium excretion [12], which is consistent with a ‘guardian’ role for 11β-HSD, it is not certain that renal 11β-HSD activities were affected. CBX prolonged the half-life of 11α-[3H]cortisol and increased the urinary excretion of free cortisol, but it had no effect on the urinary ratio of tetrahydrocortisol/tetrahydrocortisone, which is commonly used as an index of 11β-HSD activities in vivo [12]. Similarly, although Souness and Morris [13] established that the subcutaneous administration of CBX potentiated the ability of cortisol and corticosterone to decrease urinary sodium excretion in adrenalectomized rats, subsequent studies by the same group found that subcutaneous CBX had no detectable effect on renal 11β-HSD activities, despite inhibiting hepatic cortisol oxidation [14]. Moreover, the administration of CBX by this subcutaneous route also potentiated the effects of the mineralocorticoids aldosterone and 11-deoxycorticosterone on urinary sodium excretion in adrenalectomized rats [15]. Since neither of these steroids is a substrate for 11β-HSD, these findings question whether the effects of CBX on urinary electrolyte excretion can be attributed exclusively to inhibition of renal 11β-HSD activities.

It is apparent from the above studies that a direct correlation between inhibition of renal 11β-HSD activities and altered renal electrolyte excretion has not yet been demonstrated. The present study was therefore performed to determine whether graded inhibition of renal 11β-HSD activities in vivo would result in the predicted graded changes in urinary sodium and potassium excretion. To this end, adrenal intact, normal rats were infused intravenously with CBX over a broad dose range, and changes in renal 11β-HSD activities, measured directly by subsequent enzyme assays, were compared with changes in electrolyte excretion. To complement these renal studies, dose-dependent effects of CBX on the reductive and oxidative activities of hepatic 11β-HSD were similarly assessed in the same animals.

**METHODS**

**Clearance studies**

Experiments were performed on adult male Sprague-Dawley rats which had been allowed free access to water and a standard rat diet containing Na+ and K+ (140 mmol and 180 mmol/kg dry weight respectively). Animals were anaesthetized with thiopentone, 110 mg/kg, intraperitoneally (Intraval™, May and Baker Ltd, Dagenham, U.K.) and prepared surgically for whole-kidney clearance studies. Two polyethylene cannulae were placed in the right jugular vein, the bladder was catheterized, a tracheotomy was performed, and the right femoral artery and jugular vein were cannulated to allow blood sample collection and blood pressure measurement. Isotonic saline was infused intravenously at 2 ml/h throughout the experiments. One hour after the completion of surgery, [3H]inulin (2 μCi primer, 2 μCi/h) or [14C]inulin (1 μCi primer, 1 μCi/h; Amersham International, Aylesbury, Bucks, U.K.) was included in the intravenous saline infusion. After a 1-h equilibration period, all rats underwent a 1-h control period during which clearance measurements were made. The animals were then divided into four groups.

**Time-control (vehicle only) group**

Ten rats continued to receive isotonic saline at 2 ml/h throughout the remainder of the experiment (3.5 h). During the final 1.5 h (designated the experimental period), further clearance measurements were made. The 2-h interval between the control and experimental periods allowed sufficient time for any mineralocorticoid-type effect to develop after inhibition of 11β-HSD with CBX [16]. A small arterial blood sample was taken at the beginning and end of each clearance period.

**Carbenoxolone (CBX) groups**

At the end of the control period, CBX was included in the saline infusion at one of three doses: 0.06 mg/h (n = 8 rats), 0.6 mg/h (n = 8 rats) or 6 mg/h (n = 8 rats) for the remainder of the experiment (3.5 h). Again, further clearance measurements were made during the final 1.5 h. In all four groups of rats, at the end of the experiment,
the left kidney and a biopsy of the liver were removed without pre-perfusion and snap-frozen in liquid nitrogen for storage at −70 °C, and a final arterial blood sample was taken for measurement of plasma Na⁺ and K⁺.

**11β-HSD assay**

Enzyme activities in homogenates of the snap-frozen kidney and liver tissue removed from rats at the end of the clearance studies were measured using standard radiometric conversion assays.

Kidney tissue, containing approximately equal amounts of renal cortex and medulla, was homogenized in hypotonic lysis buffer (Tris, 5 mmol/l; MgCl₂, 1.5 mmol/l; EDTA, 1.5 mmol/l) [17] using a glass dounce homogenizer. Isotonicity was restored by the addition of KCl (final KCl concentration, 500 mmol/l; tissue concentration, 25 mg wet weight/ml). Phosphate-buffered saline was then added (7:1, v/v, PBS/homogenate) and the homogenate was centrifuged at 1000 g for 30 min at 4 °C. Aliquots of the supernatant (800 µl) were transferred to glass assay tubes and preincubated at 37 °C for 30 min. One-hundred microlitres of NAD⁺ or NADP⁺ (4 mmol/l; Sigma Chemical Co., Poole, Dorset, U.K.) and 100 µl of the substrate, 1,2,6,7-[³H]cortisol (final concentration, 500 nmol/l; 1 µCi/ml; Amersham International), were then added to the tubes. After 30 min incubation at 37 °C, the [³H]-labelled steroids were extracted with two volumes of ice-cold chloroform and the tubes were vortexed and centrifuged at 1000 g for 30 min at 4 °C. The aqueous phase was then discarded and the organic extracts evaporated to dryness under nitrogen at 40 °C. The [³H]-steroid residues were resuspended in ethyl acetate (containing cortisol, 1 mmol/l, and cortisone, 1 mmol/l) and samples were transferred on to aluminium-backed Silica 60 TLC plates. These were resolved in an atmosphere of 92:8 (v/v) chloroform/95% (v/v) ethanol [4].

The percentage conversion of [³H]cortisol to [³H]-cortisone was quantified using a Bioscan 200 TLC scanner (Lablogic, Sheffield, U.K.). Results were corrected for the specific activity of the substrate (1 µCi/500 pmol) and the protein concentration of the homogenate, which was measured by the protein-binding dye assay of Bradford [18]. 11β-HSD activities were then expressed as pmol cortisol oxidized to cortisone ·30 min⁻¹·mg⁻¹ protein.

Hepatic 11β-dehydrogenase activities were assayed in the presence of NAD⁺ (400 µmol/l) using a homogenate of rat liver tissue (25 mg wet weight per ml) prepared as for the renal homogenates. For the liver assays, 11-ketosteroid reductase activities (expressed as pmol cortisone reduced to cortisol ·30 min⁻¹·mg⁻¹ protein) were measured in the presence of NADPH (400 µmol/l) using 1,2,6,7-[³H]-cortisone (100 nmol/l; 0.1 µCi/ml) as substrate. The [³H]-cortisone was synthesized in house by overnight incubation of a renal homogenate at 37 °C with [³H]cortisol and NADP⁺. After resolution of the [³H]-steroids by TLC, the [³H]cortisone was eluted from the relevant area of the TLC plate using dichloromethane. This organic extract was then evaporated under nitrogen and the [³H]cortisone was resuspended in ethanol to a final specific activity of 0.1 µCi/µl.

**Analyses**

Arterial blood pressure was measured using a Druck (Groby, Leics., U.K.) pressure transducer. Urinary and plasma Na⁺ and K⁺ concentrations were measured by flame photometry (model 543, Instrumentation Laboratory, Warrington, U.K.). [³H]Inulin and [¹⁴C]inulin activities in urine and plasma samples were determined using β-emission spectroscopy after dispersal in Aquasol 2 scintillation cocktail (Canberra Packard, Pangbourne, Berks., U.K.).

**Calculations**

Mean arterial blood pressure was calculated as diastolic pressure plus one-third of the pulse pressure. Glomerular filtration rate (GFR) was calculated as the renal clearance of [³H]- or [¹⁴C]inulin. The fractional excretions of Na⁺ (FENa⁺) and K⁺ (FEK⁺) were calculated as the clearance of Na⁺ and K⁺ respectively divided by GFR.

**Statistics**

All results are presented as means ± S.E.M. Statistical comparisons were made using one-way analysis of variance to assess differences between groups within the control and experimental period. If a significant difference was shown, results were compared further with a post-hoc least significant difference test. A value of P < 0.05 was considered to be statistically significant.

**RESULTS**

Table 1 shows the body weights and plasma Na⁺ and K⁺ concentrations in the four groups of rats. There were no significant differences between the groups with respect to

<table>
<thead>
<tr>
<th>CBX</th>
<th>Vehicle</th>
<th>0.06 mg/h</th>
<th>0.6 mg/h</th>
<th>6 mg/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>260 ± 3</td>
<td>260 ± 3</td>
<td>260 ± 2</td>
<td>263 ± 3</td>
</tr>
<tr>
<td>Plasma Na⁺ (mmol/l)</td>
<td>140 ± 1</td>
<td>139 ± 1</td>
<td>140 ± 2</td>
<td>138 ± 1</td>
</tr>
<tr>
<td>Plasma K⁺ (mmol/l)</td>
<td>3.9 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>4.0 ± 0.1</td>
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these variables. In nine rats also prepared for clearance studies under thiopentone anaesthesia, measured endogenous plasma corticosterone concentrations were 0.67 ± 0.07 µM, comparable to those found in restrained unanaesthetized rats, which are 5–10 times basal concentrations in unrestrained rats (C. Kenyon, personal communication).

**Table 2** 11β-HSD (11β-dehydrogenase) activities (pmol cortisol converted to cortisone 30 min⁻¹ mg⁻¹ protein) in renal tissue taken from rats infused intravenously with CBX (0.06, 0.6 or 6 mg/h) or vehicle alone

<table>
<thead>
<tr>
<th>CBX</th>
<th>Vehicle</th>
<th>0.06 mg/h</th>
<th>0.6 mg/h</th>
<th>6 mg/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAD⁺</td>
<td>86 ± 22</td>
<td>44 ± 8*</td>
<td>20 ± 5†</td>
<td>4 ± 2†</td>
</tr>
<tr>
<td>NADP⁺</td>
<td>292 ± 72</td>
<td>182 ± 28</td>
<td>66 ± 27†</td>
<td>4 ± 1†</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. from 6 to 7 rats per group. Renal tissue was incubated with either NAD⁺ or NADP⁺. *P < 0.05, †P < 0.001 compared with corresponding vehicle value.

**Enzyme activity**

Table 2 shows the activities of 11β-HSD in renal tissue (taken at the end of the clearance studies) incubated with NAD⁺ or NADP⁺. In vivo infusion of CBX caused a dose-dependent inhibition of both NAD⁺- and NADP⁺-dependent 11β-HSD activities (assayed ex vivo), achieving ≥ 90% inhibition at a dose of 6 mg/h. NAD⁺-dependent renal 11β-HSD activity was inhibited at all CBX doses tested (Figure 1a); inhibition of NADP⁺-dependent 11β-HSD activity was statistically significant only at the intermediate and high CBX doses.

Table 3 shows the 11β-dehydrogenase and 11-ketosteroid reductase activities of 11β-HSD in hepatic tissue incubated with NADP⁺ or NADPH respectively. As in the kidney, NADP⁺-dependent cortisol oxidation by the hepatic homogenate was significantly inhibited in vivo at CBX doses of 0.6 and 6 mg/h. However, inhibition of hepatic 11-ketosteroid reductase activities was less pronounced at the lower doses of CBX and only achieved statistical significance at the highest dose (6 mg/h; Figure 1b).

**Clearance studies**

The effects of CBX on mean arterial blood pressure, GFR, the fractional excretions of Na⁺ and K⁺ and the urinary Na⁺/K⁺ ratio are shown in Figures 2, 3 and 4. In each figure, values are shown for the four groups of rats during both the control period and the experimental period. Mean arterial blood pressure was very similar in the four groups during the control period and also during the experimental period. Blood pressure fell slightly during the course of the experiment, but CBX itself had no effect (Figure 2a). There were no changes in GFR in any of the groups during the course of the experiment, and there were no differences between groups either during the control period or during the experimental period (Figure 2b).

The fractional excretion of Na⁺ (FE_{Na⁺}) was similar in the four groups during the control period, and fell in all groups during the course of the experiment. During the experimental period, however, FE_{Na⁺} was significantly lower in the group of rats given the highest dose of CBX (6 mg/h) than in the vehicle-treated group (P < 0.05).
but there was no such decrease in rats treated with the two lower doses of CBX (Figure 3a). During the control period, the fractional excretion of K⁺ (FEK⁺) was also similar in the four groups; again there was a tendency for FEK⁺ to fall during the course of the experiment, but there were no statistically significant differences between the four groups during the experimental period (Figure 3b).

There were no differences in the urinary Na⁺/K⁺ ratio between groups during the control period; in all groups this ratio tended to decrease between the control and experimental periods (Figure 4). During the experimental period, the Na⁺/K⁺ ratio in the high-dose CBX group was significantly lower than that in the vehicle-treated group (P < 0.001). However, at doses of 0.06 and 0.6 mg/h, CBX had no significant effect on the urinary Na⁺/K⁺ ratio.

### DISCUSSION

Aldosterone acts via MRs in the distal nephron to promote Na⁺ reabsorption and K⁺ secretion. As indicated in the Introduction, the MRs are non-selective between aldosterone and glucocorticoids in vitro [19], and it has been proposed that MRs in mineralocorticoid target tissues are ‘protected’ in vivo from glucocorticoid occupation by the action of 11β-HSD (specifically type 2 11β-HSD) which inactivates glucocorticoids and thus allows aldosterone to bind preferentially to the MRs [3,4]. Because the circulating concentration of glucocorticoid is normally around 100 times greater than that of aldosterone, it follows that, if the hypothesis is correct, inhibition of 11β-HSD by CBX should lead to over-stimulation of MRs in the distal nephron by endogenous glucocorticoids. This would produce a hyper-mineralocorticoid effect characterized by a decrease in urinary Na⁺ excretion and a decreased urinary Na⁺/K⁺ ratio. The present study is the first to compare direct measurements of inhibition of renal (and hepatic) 11β-HSD activities with corresponding effects on kidney function in the same rats.

### Changes in renal Na⁺ and K⁺ excretion

In order to detect renal tubular effects of 11β-HSD inhibition and adjust for the effects of changes in filtered load, we have presented fractional, rather than absolute, electrolyte excretion rates, although no significant changes in GFR were observed with any of the doses of CBX. With the highest dose (6 mg/h), the oxidative activities of both NAD⁺- and NADP⁺-dependent renal 11β-HSD isoforms were found to be inhibited by more than 90%. This degree of enzyme inhibition was associated with significant decreases in FEK⁺ and the urinary Na⁺/K⁺ ratio. This apparent increase in mineralocorticoid effect observed after the high dose of CBX is consistent with the hypothesis of a ‘guardian’ role for 11β-HSD.

It might be expected that any enhancement of mineralocorticoid effect would lead not only to a lowering of Na⁺ excretion but also to an increase in K⁺ excretion. Although there was a tendency for FEK⁺ to be raised during the experimental period after high-dose CBX as compared with the vehicle-infused group (whereas the converse was true during the control period), the apparent change did not achieve statistical significance. In this context it should be noted that Souness and Morris [13] found that high-dose corticosterone alone in adrenalectomized rats caused a kaliuresis, and that although co-administration with CBX decreased Na⁺ excretion, it did not potentiate the kaliuresis. Moreover, previous studies in humans have also found that CBX lowers Na⁺ excretion without increasing urinary K⁺ excretion [12,20]. In the latter studies, mild hypokalaemia was reported, whereas in the present study no change in plasma K⁺ concentration was observed at any dose of CBX, which might be due to the relatively short duration of these experiments. Moreover, in studies of adrenalectomized rats by Field et al. [21], the acute administration of aldosterone produced the expected increase in K⁺ secretion in perfused distal tubules, but net renal K⁺ excretion was unaffected, despite reduced Na⁺ excretion. Thus, the absence of a demonstrable kaliuretic effect of CBX in the present study, even at the highest dose, suggests that net K⁺ excretion is not a sensitive index of distal nephron MR stimulation.

### Changes in kidney enzyme activity

The major finding of the present study was that the intermediate and low doses of CBX (0.6 and 0.06 mg/h), which produced approximately 80% and 50% inhibition of renal 11β-HSD activities respectively, did not produce any corresponding changes in renal Na⁺ excretion. These results can be contrasted with those of Latif et al. [14]

| Table 3 11β-Dehydrogenase (11β-DH; pmol cortisol oxidized to cortisone 30 min⁻¹ mg⁻¹ protein) and 11-ketosteroid reductase activities (11-KSR; pmol cortisone reduced to cortisol 30 min⁻¹ mg⁻¹ protein) in hepatic tissue taken from rats infused intravenously with CBX (0.06, 0.6 or 6 mg/h) or vehicle alone |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Vehicle         | 0.06 mg/h       | 0.6 mg/h        | 6 mg/h          |
| 11/β-DH (NADPH)                | 626 ± 149       | 537 ± 93        | 94 ± 21†        | 11 ± 3†         |
| 11-KSR (NADPH)                 | 74 ± 15         | 50 ± 12         | 39 ± 15         | 12 ± 4*         |
who administered CBX subcutaneously to rats and reported an enhancement of mineralocorticoid effects of cortisol and corticosterone without inhibition of renal 11β-HSD. Despite the differences between the two studies, the factor common to both is a lack of correlation between inhibition of renal 11β-HSD activities and altered Na⁺ excretion, which accords with the situation in humans given CBX in whom no link could be found between urinary indices of 11β-HSD activities and Na⁺ excretion (see Introduction).

In the present study, we have measured 11β-HSD activities ex vivo (after in vivo exposure to CBX) in the presence of NADP⁺ and NAD⁺ in an attempt to discriminate effects on type 1 and type 2 11β-HSD activities respectively. However, we recognize that although 11β-HSD2 has an absolute requirement for NAD⁺ as cofactor, 11β-HSD1 can employ either NADP⁺ or NAD⁺ as cofactor. Consequently, cortisol oxidation in the presence of NADP⁺ can be attributed solely to 11β-HSD1, whereas oxidation in the presence of NAD⁺ will reflect the activities of both 11β-HSD isoforms. Thus, significant decreases in NAD⁺-dependent cortisol oxidation could reflect inhibition of 11β-HSD1 that may not necessarily be accompanied by inhibition of 11β-HSD2 activity in the distal nephron. Based on the NADP-dependent 11β-HSD activities, it would seem that the dose of CBX required in vivo to inhibit both renal and hepatic 11β-HSD1 activity significantly is

![Figure 2](image.png)

**Figure 2** Mean arterial blood pressure (a) and glomerular filtration rate (b) in the four groups of rats during the control and experimental periods

Values are means ± S.E.M. (n = 8–10 rats per group). There were no significant differences between the groups for either variable during either period.
0.6 mg/h. Therefore, the ability of 0.06 mg/h CBX to inhibit NAD⁺-dependent cortisol oxidation, without affecting NADP-dependent 11β-HSD activities, suggests inhibition of 11β-HSD2 in vivo at this low dose. Irrespective of specificity of action on particular renal 11β-HSD isoforms, the CBX dose of 0.6 mg/h inhibited both NAD⁺- and NADP-dependent 11β-HSD activities by approximately 80% without significantly altering net renal Na⁺ and K⁺ excretion.

Our findings suggest either that renal 11β-HSD isoforms have a very high reserve capacity, such that they can protect the MRs even when the enzymes are 80% inhibited, or that an alternative biochemical mechanism is responsible for the renal effects observed with high-dose CBX. CBX is a non-specific inhibitor of short-chain alcohol dehydrogenases [22] and it is possible that the acute effects of high-dose CBX on renal Na⁺ excretion may be attributable to the inhibition of enzymes other than 11β-HSD. For example, renal function is known to be affected by renal prostaglandins [23]; Peskar et al. [24] established that CBX can prolong the half-life of prostaglandins as a consequence of inhibition of 15-hydroxyprostaglandin dehydrogenase. Moreover, preliminary assays of [³H]prostaglandin-F2α metabolism in the renal tissue obtained in the present experiments confirm that at the highest dose of 6 mg/h, CBX inhibited renal NAD⁺-dependent prostaglandin dehydrogenase activity by 40%. At the lower doses, which had no effect on urinary electrolytes, CBX had no significant effect on the oxidation of [³H]prostaglandin-F2α [25]. This associ-
during the control and experimental periods. An alternative explanation for the anti-
natriuretic effect of high-dose CBX may involve a direct
interaction between CBX and MRs. Armanini et al. [26]
mentioned above, and hence are not attributable solely
to the inhibition of renal 11β-HSD activities. An alternative explanation for the anti-
natriuretic effect of high-dose CBX may involve a direct
interaction between CBX and MRs. Armanini et al. [26]
have reported that high-dose CBX can alter renal Na⁺/K⁺
excretion in adrenalectomized rats in the absence of
adrenal steroid replacement, and in vitro studies indicated
that at extremely high concentrations CBX could displace
the binding of [3H]aldosterone from MRs [26]. Although
the affinity of CBX for MR was only about 1/15 000 that
of aldosterone, we cannot discount the possibility in our
experiments that at the highest dose of CBX, the drug
might have had a direct action on MRs. However,
without knowledge of plasma and tissue CBX concen-
trations, this possibility remains speculative.

Changes in liver enzyme activity

With regard to the measurements of [3H]cortisone
reduction in liver homogenates, the present study is the
first to demonstrate inhibition of hepatic 11-ketosteroid
reductase activities in the liver by CBX in vivo using
direct assays of enzyme activity. These data support the
conclusion of Stewart et al. [12], which was based on the
attenuation of the rise in plasma cortisol after administra-
tion of cortisone acetate in volunteers that had taken
CBX. More importantly, we have also shown that lower
doses of CBX are unable to inhibit NADPH-dependent
hepatic 11-ketosteroid reductase activity, but retain the
ability to inhibit both renal and hepatic NADP-depen-
dent 11β-dehydrogenase activities by > 50%.

In conclusion, the ability of lower doses of CBX to
inhibit renal 11β-HSD activities by > 50% without
accompanying changes in urinary Na⁺ excretion suggests
that 11β-HSD may not be the only mechanism deter-
mining mineralocorticoid specificity in the distal
nephron. Future studies at the level of the single nephron
are needed in order to clarify the functional significance of
renal 11β-HSD activities with respect to adrenal steroid
regulation of renal electrolyte balance.

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