11β-Hydroxysteroid dehydrogenase type 1 is a predominant 11β-reductase in the intact perfused rat liver

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Abstract

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD-1), a regulator of intrahepatocellular glucocorticoid activity, is bidirectional in homogenates but catalyses 11β-reduction (regenerating glucocorticoid) in intact primary hepatocytes in culture. To examine this discrepancy at the whole-organ level, we examined 11β-HSD-1 activity in the intact bivascularly perfused rat liver. On a single pass through male rat liver, 44 ± 5% of 11-dehydrocorticosterone (11-DHC) recovered was 11β-reduced to corticosterone, whereas 10 ± 1% of corticosterone was 11β-dehydrogenated to 11-DHC. 11β-Reduction was less in female liver (21 ± 2%, $P<0{\cdot}01$) and was significantly greater with perfusion of all substrate via the portal vein (50 ± 3%) than via the hepatic artery (30 ± 2%, $P<0{\cdot}05$). 11β-Reductase activity was not saturated by 11-DHC (10^{-9}-10^{-6} M). Perfusion with carbenoxolone (CBX, 10^{-6}-10^{-3} M) did not alter 11β-reduction of 11-DHC. In contrast, pretreatment with CBX *in vivo* (10 mg/day) for 7 days inhibited 11β-reductase (19 ± 4% conversion, $P<0{\cdot}01$). Concentrations of 11-DHC in male rat plasma were 44 ± 6 nM. Thus 11β-HSD-1 is predominantly an 11β-reductase in the intact rat liver and is only inhibited by chronic administration of CBX. The substantial concentrations of plasma 11-DHC as substrate suggest that 11β-HSD-1 activity and its potential selective inhibition could modify glucocorticoid action *in vivo*.

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Introduction

Glucocorticoids regulate many metabolic and homeostatic processes and comprise a key component of the response to stress. The tissue response to glucocorticoids is modulated not only by circulating concentrations of steroids and the cellular expression of their receptors, but also by the presence of 11β-hydroxysteroid dehydrogenases (11β-HSD), which catalyse the interconversion of physiologically active glucocorticoids (cortisol, corticosterone) and their inert 11-dehydro forms (cortisone, 11-dehydrocorticosterone (11-DHC)) (Monder & White 1993, Krozowski *et al.* 1999).

11β-HSD type 1 is an NADP(H)-dependent enzyme, showing bidirectional activity in tissue homogenates and purified microsomal fractions *in vitro* (Lakshmi & Monder 1988). However, in intact cells the enzyme is usually a predominant 11β-reductase, regenerating active corticosterone from inert 11-DHC. The predominance of reductase activity in intact cells has been observed both in cell lines transfected with 11β-HSD-1 cDNA (Agarwal *et al.* 1990) and in primary cells that maintain 11β-HSD-1 activity in culture, including hepatocytes, adipose stromal cells and lung cells (Hundertmark *et al.* 1995, Jamieson *et al.* 1995, Bujalska *et al.* 1997). However, the conditions in cell culture are very different from those that pertain *in vivo*. In particular, conditions of primary culture are known to alter many differentiated functions of hepatocytes *in vivo* (Clayton & Darnell 1983).

The potential for 11β-reduction to potentiate, or for 11β-dehydrogenation to attenuate, glucocorticoid action demonstrates that the directionality of 11β-HSD-1 is crucial to its influence on corticosteroid receptor activation within tissues. 11β-HSD-1 is highly expressed in liver, and its role in glucocorticoid action in this tissue has only recently been investigated. Data are suggestive of a biological role for hepatic 11β-HSD-1 in the regulation of glucocorticoid-regulated hepatic transcripts, including those involved in glucose metabolism (Jamieson *et al.* 1999*a*). The 11β-HSD-1 knockout mouse (Kotelevtsev *et al.* 1997) shows attenuated activation of phosphoenolpyruvate carboxykinase in response to starvation and resists hyperglycaemia with stress or obesity. Inhibition of 11β-HSD-1 *in vivo* increases insulin sensitivity in humans (Walker *et al.* 1995) and decreases fasting glucose concentrations in rats (P M Jamieson & J R Seckl, unpublished observations).

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Indirect studies have supported the notion that hepatic 11β-HSD-1 is a reductase *in vivo*. The effluent of perfused cat liver has a high cortisol:cortisone ratio (Bush 1969). Similarly in humans, measurement of the cortisol:cortisone ratio by selective venous catheterisation suggests a fivefold greater value in the hepatic vein than in most other veins (Walker *et al.* 1992). In humans, cortisone administered orally is converted to cortisol on first pass through the liver and this conversion is impaired after administration of the 11β-HSD inhibitor, carbenoxolone (CBX) (Stewart *et al.* 1990, Walker *et al.* 1995). However, these indirect *in vivo* measurements may be confounded by 11β-HSD activities in other organs and do not assess the absolute activities of 11β-reductase and 11β-dehydrogenase (i.e. turnover) of 11β-HSD-1 in liver. To investigate this we have examined the metabolism of glucocorticoid across the intact perfused rat liver.

Materials and Methods

Animal procedures

Adult Wistar rats (200–250 g) were maintained under conditions of controlled lighting (lights on 0700 to 1900 h) and temperature (22 °C), with water and food available *ad libitum*. The animal procedures undertaken were all approved under the British Home Office Animals Scientific Procedures (1986) Act.

Surgical procedures

Dual liver perfusions were established. Kreb's Ringer buffer (KRB; 118 mmol/l NaCl, 3·8 mmol/l KCl, 1·19 mmol/l KH_2PO_4, 2·54 mmol/l $CaCl_2$, 1·19 mmol/l $MgSO_4.7H_2O$, 25 mmol/l $NaHCO_3$ and 0·2% glucose, pH 7·4), supplemented with 11-DHC, corticosterone or dexamethasone (all Sigma Chemical Co., Poole, Dorset, UK) (dissolved in ethanol; final concentration of ethanol in perfusate was 0·02% in all experiments), was maintained at 40 °C (equivalent to 37 °C at point of entry to the liver) and constantly bubbled with 95%O_2–5%CO_2. Rats were killed with pentobarbitone (60 mg/kg, i.p.), the abdomens opened and the vascular bundles supplying the stomach, pancreas and small intestine tied off. The hepatic artery was cannulated with a 24 G × 0·75 inch Surflo cannula (Terumo, Belgium) and 250 i.u. heparin in 2 ml KRB injected; subsequently, 2 ml/min KRB was delivered via the cannula by peristaltic pump. The inferior vena cava was cannulated posterior to the inflow of the hepatic vein using an 18 G × 45 mm Venflon cannula (Ohneda, Sweden) to allow collection of effluent. Finally, the hepatic portal vein was cannulated with a 18 G × 45 mm Venflon cannula, flushed with 250 i.u. heparin in 2 ml KRB and 2 ml/min KRB was delivered by a second peristaltic pump. The perfusion rate was increased to 7·5 ml/min through each vessel in male rats and 5 ml/min in female rats (this was the maximum flow rate the hepatic artery could accommodate in females) and outflow measured to ensure there were no leaks. These flow rates were maintained throughout perfusions except where stated. [^{3}H]11-DHC, synthesised from [^{3}H]corticosterone as described previously (Brown *et al.* 1993), [^{3}H]corticosterone (10^{-9} M) (specific activity 84 Ci/mmol; Amersham International, Aylesbury, Bucks, UK) or [^{3}H]dexamethasone (specific activity 84 Ci/mmol; Amersham International) was added to the perfusate as tracer, and the system allowed to equilibrate for 10–15 min before collection of samples (10 ml, in triplicate). After extraction into ethyl acetate, steroids were dried down under air at 55 °C, reconstituted in 600 μl mobile phase (65:35 methanol:water (HPLC grade)) and analysed by HPLC with an on-line ultraviolet absorbance detector set at 254 nm and an on-line β-counter.

Gas chromatography mass spectrometry

Concentrations of corticosterone and 11-DHC were measured in rat plasma by gas chromatography mass spectrometry. Briefly, plasma samples (2 ml, $n=6$), collected in the afternoon after decapitation of the rats and containing the internal standards [^{2}H]9-corticosterone and [^{2}H]10–11-DHC (Best *et al.* 1997), were vortexed with ethyl acetate (10 ml) and the organic phase reduced to dryness. The residue was derived and analysed as described previously (Kotelevtsev *et al.* 1997). Quantification was achieved using the ratios of the sum of the peak areas of the ions of the derived analyte to those of the derived deuterated standard.

Experimental procedures

Measurement of 11-DHC and corticosterone metabolism in perfused liver

Perfusate was supplemented with corticosterone (10^{-8} M) or 11-DHC (from 10^{-9} M to 10^{-6}M, as required) and 10 ml aliquots of effluent collected in triplicate. The products of 11-DHC or corticosterone metabolism in the liver were examined, and the proportion of steroid recovered was calculated as (c.p.m. recovered in sample/c.p.m. recovered in control perfusate) × 100%. Control perfusate had not been passed through the liver. To examine the possible influence of the route of delivery of glucocorticoids upon their metabolism, perfusate was delivered through the hepatic artery alone, the hepatic portal vein alone, or a combination of the two vessels in varying proportions in male rat livers. The standard flow rate of 15 ml/min was chosen to approximate the physiological rate of blood flow through the male rat liver (approximately 1·75 ml/min per gram

(Angus *et al.* 1995)). When flow rates were altered, the system was allowed to equilibrate for 5–10 min at each rate before further sample collection. Alterations in total flow rate (as opposed to route of delivery) did not alter 11-DHC or corticosterone metabolism over the range examined (2–20 ml/min; data not shown).

Analysis of steroid products

To identify products of 11-DHC or corticosterone metabolism, the HPLC elution profiles of steroid standards for the major hepatic metabolites of corticosterone were examined by absorbance at 254 nm (see Fig. 2). A liver perfusion was carried out in a male rat with 10^{-8} M unlabelled 11-DHC and 5×10^{-9} M [^{3}H]11-DHC in the perfusate. Radiolabelled peaks were compared with the elution profiles of the unlabelled steroid standards to identify products of 11-DHC or corticosterone metabolism (see Fig. 2). Ethyl acetate extraction does not recover water-soluble conjugated steroid products, therefore further samples were analysed for total [^{3}H]steroid recovered (unconjugated and conjugated steroid) by extraction on C18 Sep-Pak columns followed by elution in methanol (2 ml). The dried eluate was hydrolysed with β-glucuronidase and sulphatase (100 μl for 48 h at 37 °C in sodium acetate buffer (0·2 M, adjusted to pH 4·6 with acetic acid)) and separated by HPLC.

[^{3}H]Dexamethasone perfusion of liver

Male rat liver was perfused with 10^{-8} M dexamethasone and 10^{-9} M [^{3}H]dexamethasone was added to the perfusate. Samples were analysed for ethyl acetate extractable (unconjugated) and total [^{3}H]steroid recovered (unconjugated and conjugated) by extraction on C18 Sep-Pak columns followed by HPLC separation. After termination of the perfusion, liver tissue was homogenised and [^{3}H] content measured to estimate total [^{3}H]steroid bound in the liver (expressed as c.p.m. in whole liver/total c.p.m. perfused) × 100%.

CBX inhibition of 11β-HSD-1 activity

To examine the inhibition of 11β-HSD-1 by CBX in perfused liver (as opposed to *in vitro* conditions), liver perfusions were carried out in male rats with 10^{-8} M unlabelled 11-DHC and 10^{-9} M [^{3}H]11-DHC in the perfusate. After equilibration of the preparation, CBX (dissolved in KRB) was added to the perfusate at increasing concentrations from 10^{-9} M to 10^{-4} M and samples collected for analysis. When the concentration of CBX was altered, the system was allowed to equilibrate for 10 min before further collection of samples. To examine the effect of prior administration of CBX, male rats were injected s.c. with 10 mg CBX daily for 7 days before liver perfusion in which the perfusate contained no CBX.

Statistical analysis

Data were compared by ANOVA and Newman–Keuls *post hoc* test or by Student's *t*-test, as appropriate. Significance was set at $P<0{\cdot}05$. Data are expressed as mean ± S.E.M. of four to eight replicates (indicated in figure legends).

Results

Glucocorticoid metabolism and 11β-HSD-1 direction in the perfused liver

In male rats perfused with [^{3}H]11-DHC via both the hepatic artery and the portal vein (50% of perfusate via each vessel), the recovery of perfused steroid was 11 ± 1% (estimated from total radioactivity recovered in ethyl acetate extracted effluent). This was independent of flow rate (data not shown). A further 10% of steroids were recovered as conjugated products. When the liver was perfused with [^{3}H]dexamethasone, 46% of radioactivity in the perfusate was recovered as unconjugated steroids. A further 12% was recovered as conjugated steroids in the effluent, and 21% was estimated as being bound in the liver by scintillation counting. Thus approximately 80% of the dexamethasone perfused into the liver was accounted for, so the difference between total steroid recoverable when liver was perfused with [^{3}H]dexamethasone (58%) and that recoverable in the effluent when perfused with [^{3}H]11-DHC (21%) was 37% of total steroid perfused.

After perfusion with 11-DHC, 44 ± 5% of the total steroid recovered had been converted to corticosterone by 11β-reductase activity. Perfusion with 11-DHC also yielded steroid products other than corticosterone, accounting for 15 ± 3% of [^{3}H]steroid present in the effluent (Fig. 1). The remainder of recovered steroid was unaltered [^{3}H]11-DHC. Perfusion with [^{3}H]corticosterone showed that only 10 ± 1% of the total steroid recovered had been converted to 11-DHC, and 23 ± 3% was converted to other steroids (Fig. 1).

The steroid peaks identified on HPLC analysis were compared with the elution profiles of standards to identify any products (Fig. 2). The majority of steroid recovered co-eluted with authentic corticosterone. However, two consistent smaller peaks were also found, accounting for up to 30% of the total radiolabelled steroid recovered. These peaks did not co-elute with any of the steroid standards examined and so could not be identified.

As the proportion of the perfusate delivered through the hepatic artery as opposed to the portal vein was increased the conversion of 11-DHC to corticosterone decreased, whereas the percentage of [^{3}H]steroid recovered as other

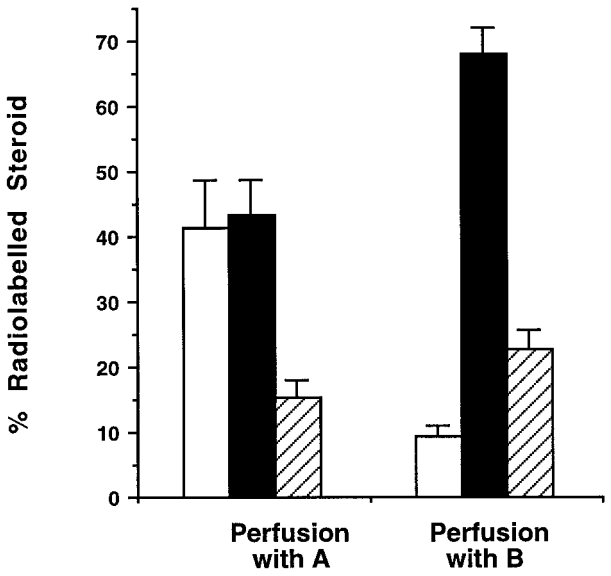

Figure 1 11β-HSD-1 activity across the intact perfused liver. Percentage of total [^{3}H]-labelled steroid recovered from the effluent of male rat livers perfused with 10^{-8} M 11-DHC (A) and 10^{-8}M corticosterone (B). □, 11-DHC; ■, corticosterone; hatching, other steroids. $n=5$.

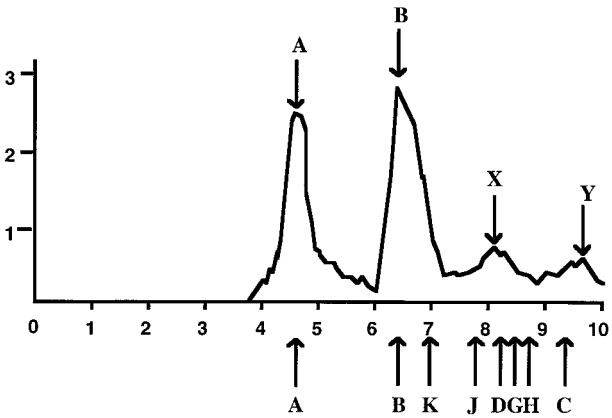

Figure 2 Products of 11-dehydrocorticosterone metabolism in the perfused liver. Representative HPLC trace of radiolabelled peaks in an extract of effluent from male rat liver perfused with [^{3}H]-11-HDC. The elution times of peaks were compared with the major hepatic metabolites of 11-DHC and corticosterone (marked by arrows on the *X*-axis) as measured by u.v. absorbance at 254 nm in order to identify the products. A, 11-DHC; B, corticosterone; C, 5α-dihydrocorticosterone; D, 5β-dihydrocorticosterone; G, 3α,5α-tetrahydrocorticosterone; H, 3β,5α-tetrahydrocorticosterone; J, 3α,5β-tetrahydrocorticosterone; K, 3β,5β-tetrahydrocorticosterone. Peaks identified were 11-DHC (27·9 ± 2·8%) and corticosterone (42·9 ± 0·9%). Peaks X (20·1 ± 1·1%) and Y (8·8 ± 0·7%) were not identified.

products (non-corticosterone, non-11-DHC steroids) was not affected by the route of delivery of 11-DHC (Table 1). The proportions of the [^{3}H]steroid products recovered when the liver was perfused with [^{3}H]corticosterone were unaffected by the route of delivery (Table 1).

Effect of substrate concentration on 11β-HSD-1 activity in the perfused liver

As the concentration of 11-DHC perfused through the liver was varied across a wide range of physiologically relevant concentrations, the percentage of steroid recovered as corticosterone did not vary significantly. Similarly, the percentage of steroid recovered as steroid products other than 11-DHC and corticosterone did not vary with the concentration of 11-DHC substrate (Fig. 3). The total recovery of [^{3}H]steroid in the effluent also did not alter (data not shown).

Sex differences in hepatic 11β-reductase

In female liver, 21 ± 2% of recovered [^{3}H]11-DHC was converted to [^{3}H]corticosterone, thus demonstrating significantly less (52% lower) hepatic 11β-reductase activity than observed in males ($P<0·05$). In contrast, a similar proportion (19 ± 3%) of steroid in the hepatic venous effluent was non-corticosterone, non-11-DHC steroids (Fig. 4).

Inhibition of 11β-HSD-1 activity by CBX

Conversion of 11-DHC to corticosterone in the intact perfused liver was not significantly inhibited by simultaneous perfusion with CBX, even at 10^{-3} M (Fig. 5). The percentage of steroid recovered as products other than 11-DHC and corticosterone and the total recovery of steroid did not change with CBX (data not shown). In contrast, 7 days of pretreatment with CBX *in vivo* significantly inhibited the conversion of 11-DHC to corticosterone (reduced by 57 ± 9%) in the intact perfused liver (Fig. 6). Again, the percentage of steroid recovered as products other than 11-DHC and corticosterone and the total recovery of steroid did not change after 7 days of pretreatment of rats with CBX.

Rat plasma corticosterone and 11-DHC concentrations

Concentrations of 11-DHC in rat plasma have not been documented and so were measured by gas chromatography mass spectrometry. Afternoon samples gave plasma corticosterone concentrations of 176 ± 41 nM and 11-DHC concentrations of 44 ± 6 nM.

Discussion

Our studies in the perfused rat liver demonstrate the predominance of 11β-HSD reduction over 11β-dehydrogenation in the intact organ. Thus, in the male rat

Table 1 Effect of route of delivery upon 11β-HSD-1 activity in the perfused liver. Percentage of [^{3}H]-labelled steroid recovered from the effluent of male rat livers perfused with 10^{-8} M 11-DHC and 10^{-8} M corticosterone as the proportion of the perfusate delivered via the hepatic artery to that delivered via the hepatic portal vein was varied from 100 to 0%. Values are means ± S.E.M., $n=5$

	Inflow via hepatic artery (%)						
	100	83	67	50	29	17	0
Outflow							
Livers perfused with 11-DHC							
11-DHC	52 ± 3	48 ± 9	45 ± 9	36 ± 7	28 ± 5	28 ± 6	24 ± 5**
Corticosterone	30 ± 2	32 ± 9	36 ± 8	38 ± 6	43 ± 5	48 ± 5	50 ± 3**
Other steroids	18 ± 4	20 ± 3	19 ± 3	26 ± 3	29 ± 4	24 ± 3	26 ± 3
Livers perfused with corticosterone							
11-DHC	10 ± 2	12 ± 3	12 ± 2	9 ± 2	9 ± 2	6 ± 2	6 ± 2
Corticosterone	69 ± 3	66 ± 5	64 ± 3	68 ± 4	70 ± 7	79 ± 7	80 ± 7
Other steroids	21 ± 2	22 ± 2	24 ± 4	23 ± 3	21 ± 6	15 ± 6	14 ± 5

**$P<0.01$ compared with 100%.

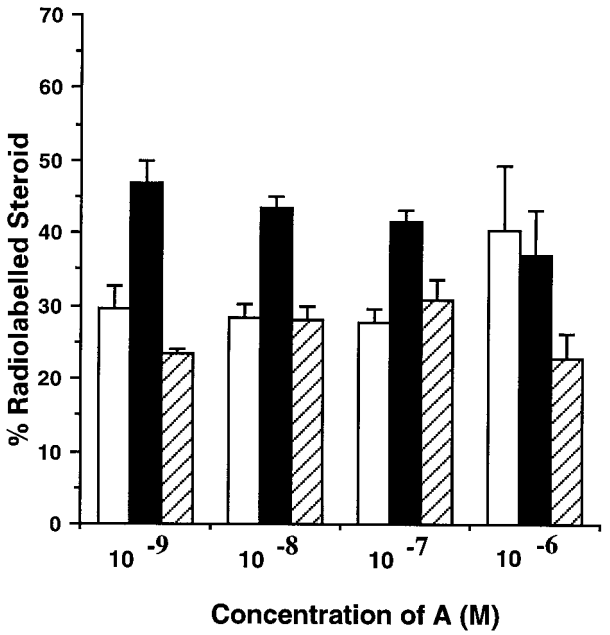

Figure 3 Effect of substrate concentration upon 11β-reductase activity in the perfused liver. Percentage of [^{3}H]-labelled steroid recovered from the effluent of male rat livers perfused with varying concentrations of 11-DHC. □, 11-DHC; ■, corticosterone; hatching, other steroids. $n=5$.

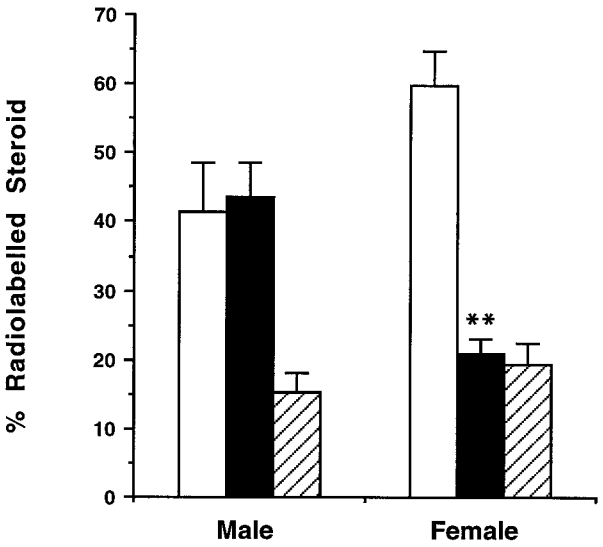

Figure 4 Sexual dimorphism of 11β-HSD-1 activity across the intact perfused liver. Percentage of [^{3}H]-labelled steroid recovered from the effluent of livers perfused with 10^{-8}M 11-DHC. □, 11-DHC; ■, corticosterone; hatching, other steroids. **$P<0.01$ compared with males. $n=5$. Control group the same as Fig. 1.

liver, the direction of 11β-HSD in the intact liver resembles more closely the findings in intact hepatocytes than those in liver homogenates. The well-documented sexual dimorphism of biochemical activity (Lax *et al.* 1978) was reflected in the *ex vivo* activity of 11β-reductase in whole liver, with female rats having approximately half the activity of males.

However, in contrast to near exclusive 11β-reductase activity in intact hepatocytes and other cells in primary culture (Hundertmark *et al.* 1995, Jamieson *et al.* 1995, Bujalska *et al.* 1997), 11β-dehydrogenation was also clearly detectable in the liver perfused *in situ*. Previous studies in humans and cats reporting predominant 11β-reduction in liver on the basis of cortisol:cortisone ratios (Bush 1969, Walker *et al.* 1992) are unlikely to have been able to detect minor 11β-dehydrogenation in the face of such predominant 11β-reduction.

There are several possible reasons for the discrepancy between intact hepatocytes and the whole perfused organ. First, the equilibrium of the 11β-HSD-1 reaction is determined by substrate and co-factor availability and product inhibition; any of these may differ *in vivo* and *in vitro*. Secondly, 11β-dehydrogenation in the perfused liver could be an experimental artefact. 11β-dehydrogenase activity is readily demonstrable in

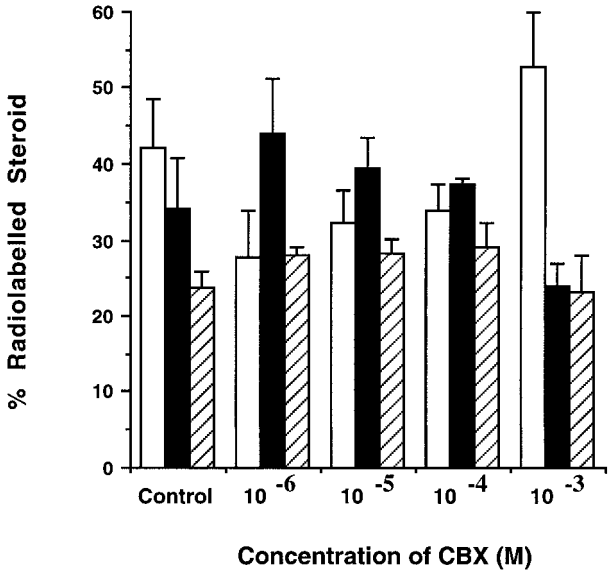

Figure 5 CBX inhibition of 11β-reductase activity in the perfused liver. Percentage of [^{3}H]-labelled steroid recovered from the effluent of male rat livers perfused with 10^{-8} M 11-DHC together with various concentrations of CBX. □, 11-DHC; ■, corticosterone; hatching, other steroids. $n=5$.

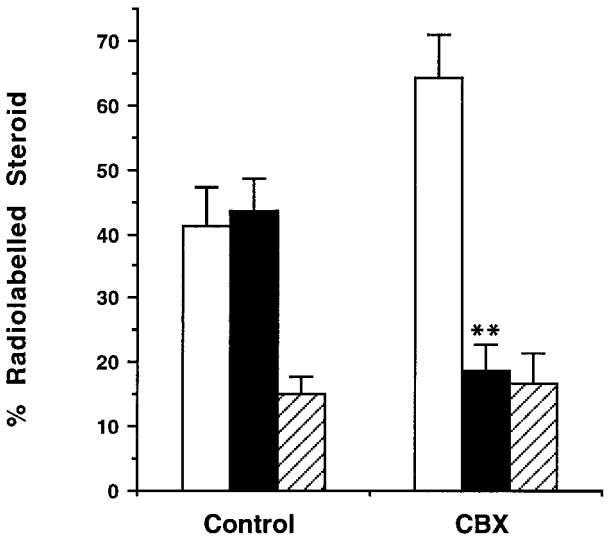

Figure 6 11β-Reductase activity in the perfused liver after pretreatment with CBX. Percentage of [^{3}H]-labelled steroid recovered from the effluent of male rat livers perfused with 10^{-8} M 11-DHC, after 7 days of pretreatment with CBX, and in controls. □, 11-DHC; ■, corticosterone; hatching, other steroids. $^{**}P<0{\cdot}01$ compared with control. $n=5$. Control group the same as Fig. 1.

homogenates of tissues or cells containing 11β-HSD-1 (Jamieson *et al.* 1995), and so in a potentially compromised tissue such as a perfused liver, 11β-HSD-1 protein may be released from dying cells and catalyse 11β-dehydrogenation. In this case, it would be expected that 11β-dehydrogenation would increase over the duration of an experiment, but this did not occur. Thirdly, the apparent bidirectionality in the whole liver may be due to the presence of another isozyme catalysing 11β-dehydrogenation. 11β-HSD-2 is the only other known 11β-dehydrogenase, and this isozyme is absent from rat liver parenchyma (Albiston *et al.* 1994). 11β-HSD-2 immunoreactivity has, however, been reported in rat bile ducts (Smith *et al.* 1997) and 11β-HSD-2 mRNA is present in vascular endothelial cells (Brem *et al.* 1998).

Approximately 80% of 11-DHC or corticosterone delivered in the perfusate was not recovered in the perfusion effluent. The concentration of steroid used in the liver perfusions was chosen as an approximation of the concentration of 'free' circulating hormone, which determines intracellular concentrations and thus the amount available for 11β-HSD-1 metabolism. The perfusate was therefore protein-free. However, this means that a large proportion of glucocorticoid in the perfusate will bind if exposed to binding proteins. Corticosteroid binding hormone (CBG) is produced primarily in the liver (Brien 1981) and corticosterone produced by metabolism of 11-DHC will be sequestered within the liver by CBG (Mendel *et al.* 1989). In addition, corticosterone will bind to intracellular glucocorticoid receptors within the liver. The difference between total steroid recoverable when liver was perfused with [^{3}H]dexamethasone and that recoverable in the effluent when perfused with [^{3}H]11-DHC was 37% of total steroid perfused. This represents an approximation of binding of [^{3}H]11-DHC and the products of its metabolism to elements within the liver that do not bind [^{3}H]dexamethasone and its products. Dexamethasone is not bound by CBG (Pugeat *et al.* 1981), but has high affinity for glucocorticoid receptors. Therefore the extra 37% of dexamethasone recovered may represent an estimate of 11-DHC metabolites associated with CBG. The proportion of dexamethasone bound within the liver (~20%) will be bound by other proteins, including glucocorticoid receptors. Other possible fates for glucocorticoids in this system include sequestration of steroids into the biliary system. After hepatic metabolism, a large proportion of glucocorticoid metabolites undergo conjugation with glucuronic acid or sulphate in order to render them more water soluble and aid excretion. Conjugated products accounted for ~10% of steroids perfused. The other products of 11-DHC and corticosterone metabolism detected by HPLC analysis remain unidentified, but are not major dihydro- or tetrahydro- metabolites. The proportions of these products did not vary with 11β-HSD-1 activity, suggesting they are the result of alternative pathways of 11-DHC metabolism.

11β-Reduction of 11-DHC increased as the proportion of substrate delivered via the hepatic portal vein increased.

Portal blood perfuses from the periphery of the lobule to the centre, whereas hepatic arterial blood perfuses from the middle of the lobule. Therefore this may represent the effect of lower perivenous oxygen tension and pH on steroid metabolising enzymes or may be due to differences in enzyme distribution. Indeed, examination of 11β-HSD-1 in human liver by immunohistochemistry revealed centrilobular localisation with no expression around the portal triads (Ricketts *et al.* 1998*b*).

Recent data are suggestive of a biological role for hepatic 11β-HSD-1 in the regulation of glucocorticoid-regulated hepatic transcripts, including those involved in glucose metabolism (see Introduction). The potential to alter gluconeogenesis/insulin sensitivity by manipulating 11β-HSD-1 pharmacologically in the liver is clearly of therapeutic interest. However, as described for 11β-HSD-2 (Monder *et al.* 1989), the concentration of CBX (the most effective inhibitor of 11β-HSD activity available at present) required to inhibit 11β-reductase in intact cells is at least 100 times that required in homogenates (Ricketts *et al.* 1998*a*). CBX was ineffective in acute perfusion studies, even in a concentration of 10^{-3} M. Metabolism of CBX or poor tissue access may reduce its efficiency *in vivo* compared with tissue homogenates. However, after chronic administration *in vivo*, CBX was a more effective inhibitor. This may be due to greater access to the site of action, perhaps saturating the metabolic capacity. Alternatively, CBX inhibits 11β-HSD-1 transcription (Whorwood *et al.* 1993), which may be its predominant mechanism of action. The data highlight the need for a potent and selective inhibitor for 11β-HSD-1 in order to dissect the function of hepatic 11β-HSD-1.

The liver clearly has a substantial capacity for 11β-reduction, as even supraphysiological 11-DHC concentrations failed to saturate hepatic 11β-reductase. In humans, administration of oral cortisone results in markedly increased plasma cortisol and a modest but detectable increase in plasma cortisone, again suggesting efficient 11β-reduction *in vivo* (Jamieson *et al.* 1999*b*). However, endogenous substrate (11-DHC) concentrations have not been described, raising the question of how much, if any, substrate is available *in vivo*. Our studies document that 11-DHC circulates at concentrations of around 50 nM in the rat, a value that accords with blood concentrations of the human equivalent, cortisone (Whitworth *et al.* 1989). 11-Keto steroids circulate largely unbound, and so 11-DHC concentrations are likely often to equal or even exceed 'free' corticosterone concentrations, which, because of sequestration (90–97%) by CBG, are in the very low nM range for much of the day. Thus intrahepatocellular regeneration of corticosterone from circulating 11-DHC may make a substantial contribution to active glucocorticoid concentrations, a theory supported by the deficient glucocorticoid-regulated gluconeogenic enzyme responses observed in 11β-HSD-1 knockout mice (Kotelevtsev *et al.* 1997).

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