Regulation of 11 β -hydroxysteroid dehydrogenase type 1 in primary cultures of rat and human hepatocytes

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Abstract

Two isozymes of the enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD) are responsible for the inter-conversion of the active glucocorticoid, cortisol in man, (corticosterone in the rodent), to the inactive 11-keto metabolite, cortisone (11-dehydrocorticosterone). We have examined the regulation of type 1 11 β -HSD (11 β -HSD1) using primary cultures of rat and human hepatocytes, both of which express only 11 β -HSD1. Only 11 oxo-reductase activity could be demonstrated in cultured hepatocytes (apparent K_m for cortisone 382 ± 43 nM in human hepatocytes, apparent K_m for 11-dehydrocorticosterone 14.6 ± 1.5 μ M in rat hepatocytes). There exists a marked discrepancy between 11 β -HSD oxo-reductase activity and 11 β -HSD1 mRNA levels in cultured human hepatocytes and human liver. Thus oxo-reductase specific activity is much higher in the cultured hepatocytes (7.2 ± 0.01 nmoles cortisol/mg/h vs 0.89 ± 0.06 for whole liver homogenates) whilst the converse is true for steady state 11 β -HSD1 mRNA levels (0.78 ± 0.02 vs 1.94 ± 0.07 in whole liver, 11 β -HSD1/18S expressed as arbitrary units).

Carbenoxolone has a significant inhibitory effect on 11 oxo-reductase activity in both rat and human hepatocytes.

However, there is clear species-specific regulation of 11 oxo-reductase activity by thyroid hormone (tri-iodothyronine (T_3)), which increases 11 oxo-reductase activity in rat hepatocytes but has no effect on activity in human hepatocytes, and progesterone which inhibits activity in human hepatocytes, but has no effect on activity in rat hepatocytes. Neither T_3 nor progesterone altered 11 β -HSD1 mRNA levels. A series of growth factors (hepatocyte growth factor, epidermal growth factor, basic fibroblast growth factor, transforming growth factor β_1) were without effect on 11 oxo-reductase activity in cultured rat hepatocytes.

In contrast to homogenates of human liver, cultured hepatocytes express only 11 β -HSD oxo-reductase activity. This is inhibited by carbenoxolone and shows species-specific regulation by T_3 and progesterone. Growth factors do not appear to regulate activity or expression of 11 β -HSD1. The discrepant enzyme activity data and 11 β -HSD1 mRNA expression in hepatocytes and whole liver could reflect unstable 11 β -HSD1 oxo-reductase activity or, alternatively, an additional 11 β -HSD oxo-reductase isoform in cultured hepatocytes.

Journal of Endocrinology (1998) **156**, 159–168

Introduction

11 β -Hydroxysteroid dehydrogenase (11 β -HSD) (EC. 1.1.1.146) is the enzyme complex responsible for the inter-conversion of the active glucocorticoids cortisol (Kendall's compound F) in man and corticosterone (compound B) in the rodent, to their hormonally inactive 11-keto metabolites, cortisone (compound E) and 11-dehydrocorticosterone (compound A) respectively (Monder & Shackleton 1984). Two isozymes of 11 β -HSD have been cloned and characterised. Type 1 11 β -HSD was originally purified from rat liver microsomes (Lakshmi & Monder 1988), and subsequently both rat and human complementary DNAs have been isolated (Agarwal *et al.* 1989, Tannin *et al.* 1991). Human type 1 11 β -HSD (11 β -HSD1) is a revers-

ible NADP/NADPH-dependent isozyme with a K_m for cortisol of 2.1 μ M and a K_m for cortisone of 0.3 μ M, suggesting that *in vivo* this isozyme behaves predominantly as an oxo-reductase, whilst type 2 11 β -HSD (11 β -HSD2) is a high affinity uni-directional dehydrogenase with a K_m for cortisol in man of 50 nM (Brown *et al.* 1993, Stewart *et al.* 1994). Type 2 11 β -HSD cDNA has also been isolated from many species including human (Albiston *et al.* 1994) and rat (Zhou *et al.* 1995).

Recent studies have shown that mutations in the gene encoding 11 β -HSD2 account for a unique form of inherited human hypertension, the syndrome of apparent mineralocorticoid excess (Mune *et al.* 1995, Wilson *et al.* 1995, Stewart *et al.* 1996). It is this type 2 isozyme of 11 β -HSD which dictates specificity upon the

mineralocorticoid receptor (MR) (Edwards *et al.* 1988). In contrast, the type 1 11 β -HSD isozyme is predominantly localized to glucocorticoid target tissues: liver, lung, gonad, and central nervous system (Whorwood *et al.* 1995). At these sites, modulation of 11 β -HSD1 activity has been shown to regulate glucocorticoid hormone action (Whorwood *et al.* 1993a, Monder *et al.* 1994, Jamieson *et al.* 1995, Rajan *et al.* 1996).

Previous studies which addressed the regulation of 11 β -HSD were largely carried out prior to the cloning of the second isozyme. *In vitro* studies evaluating the effects of thyroid hormone (tri-iodothyronine (T₃)) (Whorwood *et al.* 1993b), sex steroids (Low *et al.* 1993) and glycyrrhetic acid, the active component of liquorice (Monder *et al.* 1989) were carried out on rodent tissues or established cell lines, which are now known to express both isozymes of 11 β -HSD. A classic example of this is the rodent kidney which, in contrast to human kidney expresses abundant amounts of both 11 β -HSD types 1 and 2. In addition, clinical studies evaluating urinary cortisol metabolites have invariably been unable to dissect out alterations in the type 1 (liver) or type 2 (renal) enzyme (Stewart *et al.* 1993, Rodin *et al.* 1994).

11 β -HSD1 is now established as a crucial pre-receptor signalling pathway for the glucocorticoid receptor and the regulation of this isozyme at a tissue level will have ramifications for glucocorticoid hormone action. With this in mind we have established an *in vitro* system to analyse further the regulation of 11 β -HSD1.

Materials and Methods

Isolation and culture of hepatocytes

Primary rat hepatocytes were obtained from male Wistar rats weighing approximately 225 g. Normal human liver tissue, excess to surgical transplant requirements was obtained from the segmental graft liver programme, Queen Elizabeth Hospital, Birmingham, under guidelines approved by the local Hospital Ethical Committee. All patients were male and aged between 7 and 51 years old. Hepatocytes were isolated and maintained aseptically at 4 °C in preservation fluid (either University of Wisconsin or Euro-Collins) (Ismail *et al.* 1991). Cells were normally isolated 12 to 24 h after removal of the liver from the donor. Both rat and human hepatocytes were isolated via two-step collagenase perfusion (modification of the method developed by Seglen) (Seglen 1976, Strain *et al.* 1991). Viability of cells was assessed by trypan blue exclusion and was >85%. Percoll gradients were used in some preparations of human hepatocytes to improve cell viability.

Cells were plated at a density of 3×10^5 /ml on rat-tail collagen-coated dishes (35 mm) in 2 ml plating medium (Dulbecco's modified essential medium (DMEM) and 10% newborn calf serum (NCS) (Gibco, Paisley, Scotland)).

After approximately 1–2 h, attached cells were washed in phosphate-buffered saline (PBS) and refed with serum-free Williams E medium (Gibco) supplemented with 0.4 mM ornithine, 100 nM insulin, 100 U/ml penicillin and 100 µg/ml streptomycin. Cultures were incubated for 72 h at 37 °C in 5% CO₂/95% air and media were changed daily. Under these conditions rat hepatocytes could be cultured for up to a maximum of 7 days before they detached, and human hepatocytes could be cultured for 10–14 days. However, experiments were always performed 48–72 h after isolation, when the cells were at the peak of S-phase (Ismail *et al.* 1991). Each experiment was repeated with hepatocytes from three separate preparations, and in each case in triplicate.

Enzymatic analysis

Determination of K_m for 11 oxo-reductase in rat and human hepatocytes Primary hepatocytes were incubated for 48 h in serum-free Williams E medium, after which the cells were incubated with various concentrations of substrate ranging from 50 nM–20 µM 11-dehydrocorticosterone (with [³H]11-dehydrocorticosterone as tracer) for 10 min. Similarly human hepatocytes were incubated with various concentrations of cortisone (0.01–2.5 µM, with [³H]cortisone as tracer). [³H]11-Dehydrocorticosterone and [³H]cortisone were synthesized in-house as previously described (Shimojo *et al.* 1996).

The steroids were extracted using dichloromethane, and run on TLC plates to separate the two steroids using 8:92, ethanol:chloroform as the mobile phase. TLC plates were scanned on a Bioscan imaging detector for 10 min and the fractional conversion of 11-dehydrocorticosterone to corticosterone, or cortisone to cortisol was calculated.

Regulation studies Hepatocytes were pre-treated for 4 or 16 h with various factors, such as carbenoxolone, T₃, progesterone, and growth factors (hepatocyte growth factor (HGF), epidermal growth factor (EGF) basic fibroblast growth factor (bFGF), transforming growth factor β_1 (TGF β_1)), followed by incubation with 1 µM 11-dehydrocorticosterone for 15 min with [³H]11-dehydrocorticosterone as tracer (rat), or 0.5 µM cortisone for 1 h with [³H]cortisone as tracer (human). At these substrate concentrations and time points product formation was linear, i.e. first order kinetics were maintained. The steroids were extracted as above, and the fractional conversion of 11-dehydrocorticosterone to corticosterone, or cortisone to cortisol was calculated. Dehydrogenase activity was assessed by incubating rat hepatocytes with concentrations of 0.1, 1 and 10 µM corticosterone with [³H]corticosterone as tracer, or human hepatocytes with 0.1, 1 and 10 µM cortisol with [³H]cortisol as tracer for varying time intervals between 5 min and 16 h. No dehydrogenase activity was observed in either case.

Enzyme activity studies in liver homogenates Normal human liver tissue was homogenized and 100 μ g/ml protein were incubated in 100 mM potassium phosphate buffer (pH 7.6), containing 200 000 d.p.m. [3 H]cortisol, 0.5–2.5 μ M cortisol, and 400 μ M NADP for 1 h at 37 °C in a shaking water-bath ($n=3$, in triplicate).

Similarly, 11 oxo-reductase activity was assessed over a range of substrate concentrations (0.1–20 μ M cortisone, with [3 H]cortisone as tracer), in the presence of 400 μ M NADPH for 1 h. The steroids were extracted as above and run on TLC.

Statistical analysis

In each case experimental data are described as the mean $\pm$ s.e.m. of three replicates of three different cultures. Data were compared using analysis of variance and Dunnett's *post hoc* tests as appropriate, with significance set at $P<0.05$.

mRNA analysis

Total RNA was isolated from rat and human hepatocytes using RNazol B (AMS Biotechnology Ltd, Oxon, UK) and a single-step extraction method based on that of Chomczynski and Sacchi (1987). Northern blot analysis was performed as previously described (Whorwood *et al.* 1993a). Briefly, 20 μ g total RNA were electrophoresed in a 1.5% agarose/15% formaldehyde gel containing ethidium bromide in 1 $\times$ MOPS (morpholinopropane-sulphonic acid buffer) (Sigma Chemical Company, Poole, Dorset, UK) at 100 mA for 4 h. RNA was transferred onto a nylon (Hybond N $^+$) membrane (Amersham International, Aylesbury, Bucks, UK) and U/V cross-linked (Hoefer Cross-Linker, San Francisco, CA, USA). 18S and 28S ribosomal bands were visualised under UV light and marked on the membrane. For probe hybridizations (11 β -HSD1 and 18S cDNAs), each membrane was prehybridized at 65 °C in hybridization buffer (0.77 M sodium phosphate, 5 mM EDTA, 7% sodium dodecyl sulphate (SDS), and 100 μ g/ml denatured salmon sperm DNA, pH 7.2) followed by hybridization with the probes for 16 h at 65 °C. Membranes were washed in 2 $\times$ SSC for 20 min at room temperature, followed by 2 $\times$ SSC/1% SDS at 65 °C for 30 min and to a final stringency of 0.1 $\times$ SSC for 10 min at room temperature (1 $\times$ SSC=150 mM sodium chloride, 15 mM trisodium citrate). All membranes were exposed to Dupont Cronex X-ray film with intensifying screens at –70 °C for between 2 h and 14 days. Before rehybridization with other probes, membranes were stripped of cDNA probes by washing with 1% SDS at 70 °C for 3 h.

Probes

The cDNAs encoding rat liver type 1 11 β -HSD (1265 basepairs (bp)) (Agarwal *et al.* 1989), the human type 1

11 β -HSD (1230 bp) (Tannin *et al.* 1991) and a rat 18S ribosomal DNA (1070 bp) (Chan *et al.* 1984), were radiolabelled with [32 P]deoxy-CTP (3000 Ci/mmol; Amersham International) by oligonucleotide random priming of the excised cDNA fragment using a commercially available kit (Pharmacia, Uppsala, Sweden).

Results

Regulation studies used primary cultures of rat and human hepatocytes, both of which only convert the inactive metabolite cortisone in man, or 11-dehydrocorticosterone in the rodent, to the active form cortisol or corticosterone. In keeping with these enzyme activity results, reverse transcription (RT)-PCR studies (Whorwood *et al.* 1995) detected only type 1 11 β -HSD expression in liver; type 2 11 β -HSD was absent in liver and hepatocytes (data not shown).

Kinetic analysis of rat 11 β -HSD oxo-reductase activity revealed a low affinity enzyme. From Lineweaver-Burk plots we established the apparent K_m for 11-dehydrocorticosterone in rat hepatocytes to be 14.6 ± 1.5 μ M (mean $\pm$ s.e., $n=3$ in triplicate), and 382 ± 43 nM for cortisone in human hepatocytes (Fig. 1).

Parallel Northern blot analyses and activity studies carried out on cultured human hepatocytes and whole human liver revealed discrepant results (Fig. 2). Specific activity (nmoles cortisol formed/mg protein/h) was much higher in cultured hepatocytes (7.2 ± 0.01) compared with whole liver homogenates (0.89 ± 0.06) whilst the converse was true for 11 β -HSD1 mRNA expression (11 β -HSD1/18S, expressed as arbitrary units, 0.78 ± 0.02 for hepatocytes, 1.94 ± 0.07 for liver, $P<0.001$).

Screening studies were performed on both rat and human hepatocytes. Table 1 shows the effects of various factors on rat and human hepatocyte 11 oxo-reductase activity. As shown in Table 2, a series of putative regulatory factors including HGF, bFGF and TGF β_1 were without effect on 11 oxo-reductase activity in rat hepatocytes. Initial experiments using relatively high doses of progesterone, T $_3$ and carbenoxolone (CBX) indicated that progesterone inhibited activity in human hepatocytes but not in rat hepatocytes, whereas T $_3$ increased activity in rat, but not human hepatocytes. CBX significantly inhibited activity in both systems at 4 h pretreatment (Table 1).

The effects of T $_3$ and progesterone were further evaluated. There was an increase in 11 β -HSD1 oxo-reductase activity following pre-incubation with T $_3$ (10^{-11} – 10^{-7} M) for 16 h in rat hepatocytes (Fig. 3a). In human hepatocytes there was a dose-dependent decrease in oxo-reductase activity following incubation with progesterone for 16 h. The maximal effective dose of progesterone in this regard was 10^{-6} M (Fig. 4a). Despite these changes in enzyme activity no significant changes in rat 11 β -HSD1 mRNA levels were observed following treatment

Figure 1 Lineweaver-Burk plots showing 1/V plotted against 1/S to establish the K_m and V_{max} for 11 oxo-reductase in rat and human hepatocytes.

with thyroid hormone (Fig. 3*b*), and no significant changes were observed in human 11β-HSD1 mRNA levels following treatment with progesterone (Fig. 4*b*).

Further analysis of the effects of CBX on the inhibition of 11 oxo-reductase activity in rat hepatocytes can be seen in Table 3. At both 4 and 16 h pre-treatment there was significant inhibition of activity, but with doses of CBX greater than 10^{-5} M. There was no change in type 1 11β-HSD mRNA levels (not shown). Similar results were observed with CBX on human hepatocytes following incubation for 4 h in the presence of 1 μM cortisone (Table 3).

Discussion

Two kinetically distinct isozymes of 11β-HSD catalyse the interconversion of hormonally active cortisol to inactive

Figure 2 Northern blot showing the expression of type 1 11β-hydroxysteroid dehydrogenase mRNA in cultured human hepatocytes compared with whole human liver. Hybridization to 18S rRNA is also shown as a control. The Table beneath the Northern blot shows the specific activities (expressed as nmoles/mg/h) for both 11 oxo-reductase and dehydrogenase activity in cultured human hepatocytes and whole human liver.

cortisone (Monder & Shackleton 1984, Tannin *et al.* 1991, Brown *et al.* 1993, Albiston *et al.* 1994, Stewart *et al.* 1994). The liver, or type 1 isozyme of 11β-HSD behaves predominantly as an oxo-reductase *in vivo* generating cortisol from cortisone. This evidence comes from several sources. First, cortisol/cortisone affinity studies carried out on the expressed human 11β-HSD isozyme reveal a lower K_m for cortisone than cortisol (Stewart *et al.* 1994). Secondly, selective venous catheterisation studies indicate low cortisone but high cortisol concentrations within the hepatic vein (Walker *et al.* 1992), in keeping with the observation that cortisone, taken by mouth, is rapidly converted to cortisol, with little or no change in circulating cortisone concentrations in the peripheral plasma (Stewart *et al.* 1990). Finally, recent experiments similar to those conducted herein on cultured rat hepatocytes have indicated high amounts of 11 oxo-reductase activity, with little or no dehydrogenase activity in intact cells (Jamieson *et al.* 1995).

The liver is known to express high levels of glucocorticoid receptors. Glucocorticoid receptors have 10–40 times lower affinity for cortisol compared with mineralocorticoid receptors which are not very abundant in the liver, and hepatic 11β-HSD is thought to be required to ensure adequate exposure of cortisol to these low affinity glucocorticoid receptors (Walker *et al.* 1995). Thus the presence of a 11β-HSD oxo-reductase ensures conversion of cortisone to cortisol and, therefore, adequate exposure to the glucocorticoid receptor. Primary cultures of hepatocytes are an excellent model to use to study the regulation of this enzyme (Jamieson *et al.* 1995) as they

Table 1 Effect of various factors on 11 oxo-reductase activity in rat and human hepatocytes after 4 and 16 h incubation. Results are means $\pm$ s.e. ($n=4$) and are expressed as percentage conversion of either A to B (11-dehydrocorticosterone to corticosterone) (rat hepatocytes) or E to F (cortisone to cortisol) (human hepatocytes) relative to control

	Percentage conversion A to B	
	4 h	16 h
Rat		
Control	100.00 $\pm$ 5.59	100.00 $\pm$ 4.54
EGF (10^{-8} M)	101.56 $\pm$ 1.85	95.83 $\pm$ 4.93
CBX (10^{-5} M)	57.14 $\pm$ 12.45**	82.69 $\pm$ 6.52
Progesterone (2×10^{-5} M)	101.19 $\pm$ 1.50	105.34 $\pm$ 4.48
T ₃ (10^{-7} M)	110.69 $\pm$ 4.48	127.99 $\pm$ 3.29**
Forskolin (10^{-5} M)	106.33 $\pm$ 0.77	106.37 $\pm$ 0.26
Percentage conversion E to F		
	4 h	16 h
Human		
Control	100.00 $\pm$ 3.92	100.00 $\pm$ 9.13
EGF (10^{-8} M)	103.34 $\pm$ 3.91	105.86 $\pm$ 2.60
CBX (10^{-5} M)	64.17 $\pm$ 1.01**	70.69 $\pm$ 1.21
Progesterone (2×10^{-5} M)	27.39 $\pm$ 1.18**	36.02 $\pm$ 1.72**
T ₃ (10^{-7} M)	94.06 $\pm$ 2.57	109.95 $\pm$ 1.18
Forskolin (10^{-5} M)	107.45 $\pm$ 0.59	107.13 $\pm$ 2.24

** $P<0.01$ compared with control.

Table 2 The effect of HGF (1, 10, 50 and 100 ng/ml), bFGF (10 and 40 ng/ml) and TGF β_1 (1 and 10 ng/ml) on 11 oxo-reductase activity in cultured rat hepatocytes. Results are means $\pm$ s.e. ($n=3$) and are expressed as percentage conversion of A to B (11-dehydrocorticosterone to corticosterone) relative to control

Growth factor	Dose	Percentage conversion A to B
Control		100.00 $\pm$ 2.42
HGF	1 ng/ml	113.60 $\pm$ 3.48
	10 ng/ml	116.76 $\pm$ 2.91
	50 ng/ml	105.94 $\pm$ 4.02
	100 ng/ml	102.16 $\pm$ 5.92
bFGF	10 ng/ml	108.15 $\pm$ 1.88
	40 ng/ml	107.23 $\pm$ 2.04
TGF β_1	1 ng/ml	101.05 $\pm$ 3.00
	10 ng/ml	103.97 $\pm$ 3.04

$P>0.05$ in all experiments.

represent a pure source of type 1 11 β -HSD as determined by RT-PCR studies. No type 2 11 β -HSD is present within these cells. In contrast to the earlier studies of Jamieson *et al.* (1995), our experimental model involved both rat and human hepatocytes cultured on collagen-coated dishes in serum-free conditions. Experiments were performed within 72 h after cell isolation, which precludes overgrowth of other cell types and increases the likelihood that the hepatocytes themselves would deviate less radically from their physiological state within the animal than

in longer-term cultures, where a progression of adaptive changes to *in vitro* conditions could be a possibility (Bissell & Guzelian 1980).

11 Oxo-reductase activity in rat hepatocytes was unaffected by cAMP (forskolin) and the growth factors HGF, bFGF and TGF β_1 . cAMP regulates related hydroxysteroid dehydrogenases including 3 β -HSD, 17 β -HSD (Tremblay & Beaudoin 1993) and the type 2 isozyme of 11 β -HSD (placenta but not kidney) (Pasquarette *et al.* 1996). Both HGF and TGF β_1 have profound effects on

hepatocyte growth and DNA synthesis (Braun *et al.* 1988, Strain *et al.* 1991), but in our rat hepatocyte culture system had no effect on 11 β -HSD1 activity or expression and, by inference, no effect on glucocorticoid availability *in vivo*.

Previous studies have shown that CBX and glycyrrhetic acid (GE) exert an inhibitory effect on 11 β -HSD (Monder *et al.* 1989, Stewart *et al.* 1990, Whorwood *et al.* 1993a) but this is the first time that CBX has been shown to inhibit hepatic 11 oxo-reductase *in vitro*. High doses of CBX (10^{-4} M) were required to inhibit 11 β -HSD1 oxo-reductase activity, in keeping with our earlier studies on the expressed 11 β -HSD1 isozyme (Bujalska *et al.* 1997). Despite inhibition of activity, 11 β -HSD1 messenger RNA levels did not change, suggesting competitive inhibition. We have previously demonstrated that the related compounds glycyrrhizic and glycyrrhetic acids do inhibit 11 β -HSD1 mRNA levels *in vivo* and *in vitro* (Monder *et al.* 1989, Whorwood *et al.* 1993a), but after culture for 24 h. There is no doubt that these liquorice derivatives are competitive inhibitors of the 11 β -HSD proteins and more prolonged exposure of the hepatocytes to CBX may have resulted in an inhibition of mRNA levels. Clinically, the inhibition of hepatic 11 β -HSD1 activity may explain why liquorice and its derivatives do not cause such a marked increase in the ratio of urinary cortisol/cortisone metabolites as seen in apparent mineralocorticoid excess (Stewart *et al.* 1990), the hypertensive condition caused by defective 11 β -HSD2 activity. CBX has also been shown to increase hepatic insulin sensitivity, consistent with a fall in intra-hepatic glucocorticoid activation following inhibition of hepatic 11 oxo-reductase activity (Walker *et al.* 1995).

The experiments with T₃ revealed a discrepant action on rat and human 11 β -HSD1, with an increase in oxo-reductase activity in rat hepatocytes, but not in human hepatocytes. Previous experiments with T₃ were carried out prior to the knowledge of two discrete 11 β -HSD isozymes, and invariably studied only dehydrogenase activity. 11 β -HSD dehydrogenase activity was decreased in rats treated with thyroid hormone *in vivo*, but T₃ had no effect on activity when added directly to homogenates *in vitro* (Koerner & Hellman 1964, Whorwood *et al.* 1993b), suggesting an indirect effect. In man, there is compelling data from urinary steroid measurements and plasma cortisol half-life studies that T₃ does alter 11 β -HSD1 activity (increased reductase activity) (Hellman *et al.* 1961, Zumoff *et al.* 1983), but we could not demonstrate this in our culture system. It is possible that a more prolonged exposure to T₃ is required in man,

or that the effect of T₃ is dependent upon secondary 'circulating' factors, as suggested in earlier publications (Whorwood *et al.* 1993a).

This species-specific regulation was also true for progesterone which inhibited human, but not rat 11 oxo-reductase activity. The minimal effective dose was in the micromolar range, a concentration which is clearly seen in stimulated human ovarian cycles, and compares favourably with doses used to evaluate 11 β -HSD regulation in human granulosa-lutein cells (Clarke *et al.* 1995). Progesterone is known to act as a glucocorticoid agonist, and it is possible that its inhibition of 11 β -HSD oxo-reductase activity within hepatocytes actually prevents tissue glucocorticoid excess at times of high circulating concentrations. The explanation for the differences between rat and human systems, however, is unclear. It is also of interest to note that progesterone markedly induces expression of both the type 1 and type 2 isozymes of 17 β -HSD (Casey *et al.* 1994, Poutanen *et al.* 1995), one of the closest 'relatives' to 11 β -HSD in the short-chain alcohol dehydrogenase superfamily (Krozowski 1992).

11 β -HSD1 mRNA levels were detectable in RNA from hepatocyte cultures, though there is reduced expression compared with whole human liver. The specific activity data showed abundant oxo-reductase activity within cultured hepatocytes, but no dehydrogenase activity was detectable. This is very much in contrast to activity in liver homogenates where there is much less oxo-reductase activity, and more dehydrogenase activity, but it does emphasise the point made by others that in an intact cell system 11 β -HSD1 is a reductase (Jamieson *et al.* 1995). In homogenates *in vitro* the enzyme can be driven in either direction with addition of co-factor, but for physiological regulation studies we would argue that intact cell systems should be used.

Notwithstanding this there appeared to be a discrepancy between the levels of 11 oxo-reductase activity and 11 β -HSD1 mRNA levels in cultured hepatocytes compared with whole liver. Thus, despite impressive oxo-reductase activity in cultured hepatocytes, the converse was seen for 11 β -HSD1 mRNA levels. One explanation for this discrepancy could be that hepatocytes in culture no longer perform *de novo* synthesis of 11 β -HSD1 and the mRNA and/or protein present is that which is present when the hepatocytes were first isolated. This seems unlikely as we can demonstrate high levels of oxo-reductase activity for up to 14 days in primary culture. A further explanation is that the 11 β -HSD1 oxo-reductase activity is highly unstable in homogenates *in vitro*. One

Figure 3 (a) Effects of 10^{-7} – 10^{-11} M T₃ on 11 oxo-reductase activity in rat hepatocytes. The upper panel represents pre-treatment for 4 h, and the lower panel represents pre-treatment for 16 h. Results are expressed as percentage conversion of A to B (11-dehydrocorticosterone to corticosterone). * $P < 0.05$, ** $P < 0.01$ compared with control. (b) Northern blot showing the expression of type 1 11 β -hydroxysteroid dehydrogenase mRNA in cultured rat hepatocytes treated with various doses of T₃ (10^{-7} – 10^{-11} M). Hybridization to 18S rRNA is also shown as a control.

Figure 4 (a) Effects of 16 h pre-incubation with progesterone (10^{-9} – 2×10^{-5} M) on 11 oxo-reductase activity in human hepatocytes. Results are means $\pm$ s.e., $n=3$ and are expressed as percentage conversion of E to F (cortisone to cortisol). * $P<0.05$, ** $P<0.01$ compared with controls. (b) Northern blot showing the expression of type 1 11β-hydroxysteroid dehydrogenase mRNA in cultured human hepatocytes treated for 16 h with various doses of progesterone (10^{-6} – 2×10^{-5} M). Hybridization to 18S rRNA is also shown as a control.

could also speculate that the discrepant activity/mRNA data reflects the presence of another 11β-HSD isozyme, an isozyme which acts *in vivo* as a pure reductase. In keeping with this suggestion, several patients have been described with a ‘polycystic ovary syndrome’-like phenotype, who

show a defect in the conversion of cortisone to cortisol (Phillipou & Higgins 1985) but who do not have a mutation in the 11β-HSD1 gene (Nikkila *et al.* 1993). We conclude that the hepatic isozyme of 11β-HSD is not regulated by EGF, bFGF, HGF or TGFβ₁. CBX

Table 3 Effects of carbenoxolone (10^{-4} – 10^{-7} M) on 11 oxo-reductase activity in rat hepatocytes after 4 and 16 h pre-treatment. Results are means $\pm$ S.E. ($n=3$) and are expressed as percentage conversion of A to B (11-dehydrocorticosterone to corticosterone) relative to control. Also shown is the effect of carbenoxolone (10^{-5} – 10^{-8} M) on 11-oxo-reductase activity in human hepatocytes when incubated with 1 μ M E (cortisone) for 4 h. Results are means $\pm$ S.E. ($n=3$) and are expressed as percentage conversion of E to F (cortisone to cortisol) relative to control

Percentage conversion A to B		
	4 h	16 h
Rat		
Control	100.00 $\pm$ 6.99	100.00 $\pm$ 2.20
10^{-4}	26.65 $\pm$ 14.01**	22.61 $\pm$ 4.09**
10^{-5}	61.40 $\pm$ 14.19*	84.33 $\pm$ 5.58
10^{-6}	106.30 $\pm$ 9.57	104.70 $\pm$ 1.83
10^{-7}	121.37 $\pm$ 0.12	105.13 $\pm$ 0.41
Human		
Control	100.00 $\pm$ 1.06	
10^{-5}	65.03 $\pm$ 1.87**	
10^{-6}	84.72 $\pm$ 3.46*	
10^{-7}	99.34 $\pm$ 4.63	
10^{-8}	97.05 $\pm$ 0.28	

* $P<0.05$, ** $P<0.01$ compared with control.

inhibits 11 oxo-reductase activity within both rat and human hepatocytes. T₃ increases 11 oxo-reductase activity in rat hepatocytes but not in human hepatocytes. The reverse is true for progesterone, i.e. no effect is seen in rat hepatocytes but activity is significantly inhibited in human hepatocytes. Our studies suggest that these effects are not mediated at a pre-translational level. There is a marked discrepancy between 11 oxo-reductase activity and 11 β -HSD1 mRNA expression in cultured hepatocytes versus whole liver, and whilst this may reflect another isozyme of 11 β -HSD, this requires further investigation. These results suggest that there is clear species-specific regulation of this isozyme of 11 β -HSD in hepatocytes.

Acknowledgements

The authors would like to thank Mr Adrian Koegh for help with human hepatocyte preparations, Miss Kaljit Bratch for assistance in rat hepatocyte preparations, and Mrs Malgorzata Wilczynska for assistance in typing the manuscript.

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Received 18 February 1997

Revised manuscript received 10 June 1997

Accepted 3 September 1997