Characterization of 11β-hydroxysteroid dehydrogenase activity and corticosteroid receptor expression in human osteosarcoma cell lines

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Abstract

Studies in vitro and in vivo have shown that corticosteroids play an important role in bone physiology and pathophysiology. It is now established that corticosteroid hormone action is regulated, in part, at the pre-receptor level through the expression of isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD), which are responsible for the interconversion of hormonally active cortisol to cortisone. In this report we demonstrate 11β-HSD activity in human osteoblast (OB) cells. Osteosarcoma-derived OB cell lines TE-85, MG-63 and SaOS-2 and fibrosarcoma Hs913T cells express the type 2 isoform of 11β-HSD, as determined by reverse transcription polymerase chain reaction (RT-PCR) and specific enzyme assays. Enzyme activity was shown to be strictly NAD dependent with a $K_m$ of approximately 71 nM; 11β-HSD type 1 mRNA expression and enzyme activity were not detected. All four cell lines expressed mRNA for the glucocorticoid receptor (GR) and mineralocorticoid receptor, but specific binding was only detectable with radiolabelled dexamethasone ($K_d$=10 nM) and not aldosterone. MG-63 cells had two to three times more GR than the other OB cells, which correlated with the higher levels of 11β-HSD 2 activity in these cells. In contrast to the osteosarcoma cell studies, RT-PCR analysis of primary cultures of human OB cells revealed the presence of mRNA for 11β-HSD 1 as well as 11β-HSD 2. However, enzyme activity in these cells remained predominantly oxidative, i.e. inactivation of cortisol to cortisone (147 pmol/h per mg protein at 500 nM cortisol) was greater than cortisone to cortisol (10:3 pmol/h per mg protein at 250 nM cortisone). Data from normal human OB and osteosarcoma cells demonstrate the presence of an endogenous mechanism for inactivation of glucocorticoids in OB cells. We postulate that expression of the type 1 and type 2 isoforms of 11β-HSD in human bone plays an important role in normal bone homeostasis, and may be implicated in the pathogenesis of steroid-induced osteoporosis.


Introduction

Increased circulating levels of glucocorticoids, as seen for example in patients with Cushing’s syndrome and those treated with exogenous corticosteroids, are associated with severe osteopenia leading to the development of osteoporosis in both men and women (Adinoff & Hollister 1983, Lukert & Raisz 1990, Canalis 1996). This may occur as a result of direct inhibitory effects on osteoblast (OB) bone formation (Canalis 1983) or by indirect stimulation of parathyroid hormone levels and induction of osteoclast activity (Raisz & Kream 1983). Despite this, it is evident that glucocorticoids also play an important role in the regulation of normal bone turnover. While pharmacological doses of glucocorticoids appear to have inhibitory actions on bone formation, studies in vitro have shown that physiological doses of glucocorticoids are required for normal OB differentiation (Leboy et al. 1991, Shalhoub et al. 1992). This dichotomy of response to glucocorticoids is reflected by both transactivation and transrepression effects, mediated via the glucocorticoid receptor (GR) (Delany et al. 1994). Genes such as those for alkaline phosphatase (ALP), osteocalcin (OC), osteopontin and type I collagen may be either down- or up-regulated by glucocorticoids (Leboy et al. 1991, Delany et al. 1995). This appears to be dependent on the developmental stage of the OB phenotype (Pockwinse et al. 1995), and may involve regulation of local factors such as insulin-like growth factor-I or insulin-like growth factor-binding proteins (Centrella et al. 1991, Delany et al. 1994, Chevalley et al. 1996).

The equilibrium between positive and negative effects of glucocorticoids on bone is therefore likely to be determined by a range of factors, including GR expression and ligand availability. Several previous studies have demonstrated the expression of GR in OB and
OB-like cells (Chen et al. 1986, Masuyama et al. 1992, Subramaniam et al. 1992). A more recent report has suggested that the levels of this receptor may vary according to the differentiation status of OBs (Sutherland et al. 1995). However, it is clear from studies in vitro and in vivo that an equally important influence on the positive and negative effects of glucocorticoids is the concentration of hormone which is available to the GR (Lukert & Raisz 1990, Scutt et al. 1996). Indeed, in vivo, it is possible to determine a direct relationship between the cumulative dose of glucocorticoid used therapeutically and bone loss/fracture incidence (Adinoff & Holister 1983, Lukert & Raisz 1990, Canalis 1996). A further pre-receptor factor in the analysis of corticosteroid hormone action occurs at an autocrine level through the action of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), which is responsible for the interconversion of hormonally active cortisol and inactive cortisone (White et al. 1997). Two isoforms of 11β-HSD have been cloned and characterized. The type 1 isoform (11β-HSD 1), is a low affinity NADPH-dependent enzyme with predominant reductase activity converting cortisone to cortisol (Tannin et al. 1991). In contrast, 11β-HSD 2 is a high affinity, NAD+-dependent dehydrogenase catalysing the inactivation of cortisol to cortisone (Albiston et al. 1994, Stewart et al. 1994). Each enzyme has been suggested to play a discreet role in modulating corticosteroid responses. The type 2 isoform is found primarily in mineralocorticoid target tissues such as kidney and colon, and acts in a protective fashion by limiting the non-selective occupation of the mineralocorticoid receptor (MR) by cortisol (Edwards et al. 1988). Congenital deficiency of 11β-HSD 2 results in a form of mineralocorticoid hypertension (the syndrome of apparent mineralocorticoid excess (Funder et al. 1988, Milford et al. 1995)), with bone disease being an ill-understood feature of the disease (Batista et al. 1986). Activity of the type 1 isoform appears to correlate with GR expression (Whorwood et al. 1992), and appears to regulate access of glucocorticoid to the GRs in tissues such as liver (Jamieson et al. 1995), gonad (Monder et al. 1994), pituitary (Sakai et al. 1992) and adipose tissue (Bujalska et al. 1997a). The aim of this study was to characterize the expression and activity of 11β-HSD in vitro in human OB cells, and to contrast this with the expression of corticosteroid hormone receptors.

Materials and Methods

Cell culture

Human osteosarcoma cells HOS TE-85 (TE-85) and MG-63, and the fibrosarcoma cells Hs91T were maintained in Minimum Essential Medium containing Earle’s salts and 2 mM l-glutamine (Life Technologies, Paisley, Strathclyde, UK) supplemented with 10% fetal calf serum (FCS) and 1% non-essential amino acids (Life Technologies). Human osteosarcoma cells SaOS-2 were maintained in RPMI 1640 medium containing 2 mM l-glutamine (Life Technologies) supplemented with 10% FCS. Experimental cultures were grown to between 80 and 100% confluence in 75 cm² flasks.

Human OB-like cell cultures were produced using a minor modification of the technique described by Beresford et al. (1984).Briefly, trabecular bone fragments were obtained from the neck region of the femora of one male (64 years old) and one female (74 years old) subject undergoing femoral osteopathy. Samples were washed in Hanks’ balanced salt solution (HBSS) to remove non-adherent marrow-associated cells, which were discarded. The remaining trabecular bone fragments were incubated with trypsin at pH 7·6 (2·5 mg/ml) and agitated for 10 min at 37 °C. Marrow and stromal cells released during digestion were removed by washing in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% FCS and were discarded. Bone fragments were then transferred to a solution of collagenase (1 mg/ml) in HBSS and agitated for 3 h at 37 °C. After washing in HBSS the enzymatically treated bone fragments were transferred to 75 cm² flasks containing DMEM supplemented with 10% FCS, penicillin (50 U/ml) and streptomycin (50 µg/ml) for culture. OB cells were obtained by outgrowth from the bone fragments over a period of at least 14 days and were obtained in the absence of exogenously added glucocorticoids. Primary cultures of cells used experimentally had reached approximately 80% confluence and had no sub-sequent passage. Cultures produced using this methodology are heterogeneous for cell differentiation state but typically contained at least 40% cells positive for ALP.

RNA extraction

RNA was prepared from 80% confluent cells using a single-step extraction method (RNAzol B RNA isolation kit, AMS Biotechnology Ltd, Witney, UK) according to the manufacturer’s protocol. mRNA was enriched from total RNA using a PolyAtract mRNA isolation system III (Promega, Madison, WI, USA) following the manufacturer’s protocol.

Reverse transcription polymerase chain reaction (RT-PCR) analysis

Reverse transcription of RNA was performed using a Promega reverse transcription system. Briefly, 1 µg total RNA and 0·5 µg oligo(dT)15, in a final volume of 10 µl, were incubated at 70 °C for 5 min. Primer extension was then performed at 42 °C for 60 min following the addition of reaction buffer containing 50 mM Tris–HCl (pH 8·3), 50 mM KCl, 10 mM MgCl2, 10 mM dithiothreitol and 0·5 mM spermidine, 1 mM of each dNTP, 80 U RNAsin ribonuclease inhibitor and 50 U avian myeloblastosis
virus (AMV) reverse transcriptase in a final volume of 50 µl. A 4 µl aliquot of this reaction was used in subsequent PCR reactions.

11β-HSD isoenzyme RT-PCR This was carried out as described previously (Whorwood et al. 1995) using the following primers: 11β-HSD 1, sense 5‘ CTGAGTCG GATGGCTTTTTATG 3‘, antisense 5‘ ACTTGCTTG CAGAATTAG 3‘. Amplification of a 571 bp fragment was performed using an initial denaturation step of 95 °C followed by 30 cycles of 94 °C (1 min); 50 °C (1 min); 72 °C (1 min). A final elongation step at 72 °C for 7 min was also included. 11β-HSD 2 primers were: sense 5‘ ACCGTATTGAGATTTAAGC 3‘, antisense 5‘ TCAGTACTGTCTTGAAGC 3‘. Amplification of the 477 bp fragment included a denaturation step followed by 30 cycles of 94 °C (1 min); 48 °C (1 min); 72 °C (2 min). A final elongation step at 72 °C for 7 min was included.

MR RT-PCR Analysis of MR mRNA expression was carried out using the following primers: sense 5‘ AACTTG GCCTCTTGAGGACC 3‘, antisense 5‘ AGAATTC CAGCAGTGCTCTGAAGC 3‘. PCR reactions, to amplify a 450 bp fragment, were set up as for 11β-HSD 1 and 2, but with 0·5 mM MgCl2 (final concentration) and 30 amplification cycles of 94 °C (1 min); 54 °C (1 min); 72 °C (2 min).

GR RT-PCR Analysis of GR mRNA expression was carried out using the following primers: sense 5‘ TCGAC CAGTGTTCAGAGAAC 3‘, antisense 5‘ TTTCG GAACCAACGGGAATTG 3‘. Amplification of a 693 bp fragment was performed using an initial denaturation cycle of 95 °C (2 min); 55 °C (1 min); 72 °C (1 min), followed by 30 cycles of 94 °C (1 min); 55 °C (1 min); 72 °C (1 min). A final elongation step of 72 °C for 5 min was included.

β-Actin RT-PCR β-Actin was used as a control in the RT-PCR. Primers were: sense 5‘ GTCACCAACT GGGACGACA 3‘, antisense 5‘ TGGCCATCTCTTG GCTCGA 3‘. PCR reactions were set up, as for the 11β-HSD isoenzymes, but with 0·75 mM MgCl2 and 0·75 µM of each primer (final concentration). Amplification conditions of the 468 bp fragment included an initial denaturation step of 95 °C followed by 30 cycles of 94 °C (1 min); 60 °C (1 min); 72 °C (2 min). A final elongation step of 72 °C for 7 min was also included.

11β-HSD activity assays

Whole cell assays Osteosarcoma cells were seeded in six-well plates at a density of 400 000 cells/well and allowed to reach 80% confluence. The medium was replaced with serum-free medium 2 h before incubation with various concentrations of corticosteroids. Dehydrogenase activity (cortisol to cortisone conversion) was assessed using 10 nM to 2 µM unlabelled cortisol (Sigma Chemical Co., Poole, Dorset, UK) diluted in serum-free medium and tracer amounts (1·5 nM) of [3H]cortisol (specific activity 78·4 Ci/mmol, NEN, Boston, MA, USA) at 37 °C for 6 h. Conversion of cortisone to cortisol was analysed by incubating cells with 0·25 µM cortisone and tracer amounts of [3H]cortisone (50 000 c.p.m.) synthesized in house as described previously (Bujalska et al. 1997b). Primary cultures of human OB cells were initiated as described above, grown to partial confluence and then incubated with glucocorticoid substrates as described above. After incubation, steroids were extracted from the medium with 10 volumes of dichloromethane, separated by thin-layer chromatography (TLC) with chloroform: ethanol (92:8) as a mobile phase and the fractional conversion of cortisol to cortisone or cortisone to cortisol calculated after scanning analysis using a Bioscan 2000 radioimaging detector (Bioscan, Washington DC, USA). Following removal of culture medium for enzyme assay, cell monolayers were lysed in 1 ml water for subsequent protein assays. Total protein in each well was determined using a standard protein assay reagent (Bio-Rad, Hemel Hempstead, Herts, UK), and enzyme activities were expressed as pmol/h per mg protein. All assays were carried out in quadruplicate and data are reported as the mean ± s.d.

Cell lysate studies The co-factor dependency of 11β-HSD activity in osteosarcoma cells was assayed using lysates of MG-63 cells. After harvesting, cells were resuspended in ice-cold 0·1 M phosphate buffer pH 7·5 and the suspensions disrupted by sonication. Protein concentration was then determined as described above. Aliquots (500 µg) of lysate were diluted to 500 µl with 0·1 M phosphate buffer (pH 7·5) and incubated for 1 h at 37 °C under the following conditions: (i) lysate+60 nM cortisol with tracer alone; (ii) lysate+60 nM cortisol with tracer+200 µM NADP; and (iii) lysate+60 nM cortisol with tracer+200 µM NAD. After incubation, steroids were extracted from the medium with 10 volumes of dichloromethane and separated by TLC as described above for whole cell assays.

Northern blot analysis Polyadenylated [poly(A)+] mRNA (1 µg/lane) was loaded onto a denaturing 1·5% formaldehyde–agarose gel and resolved by electrophoresis (100 V, 3–4 h) before blotting onto nylon filters (Hybond N+, Amersham International, Amersham, Bucks, UK) overnight. After fixation by UV irradiation, filters were pre-hybridized (5 h) and hybridized (18 h) at 65 °C to 32P-labelled cDNA probes in a modified Church–Gilbert buffer containing 0·77 M sodium phosphate pH 7·2 (0·2 M NaH2PO4·H2O+0·58 M
Na$_2$HPO$_4$), 5 mM EDTA, 7% SDS (w/v) and 100 µg/ml denatured salmon sperm DNA. GR and MR cDNA probes were generated by RT-PCR amplification (as described above) and isolated from low melting point agarose gels. These fragments together with a cDNA probe for β-actin were then labelled by random priming (Pharmacia, Milton Keynes, Bucks, UK) or nick translation (β-actin) (Amersham) with [32P]deoxy-CTP (3000 Ci/mmol; Amersham). Filters were washed to a final stringency of 0·3× SSC (standard sodium citrate)+0·1% SDS at 55 °C prior to autoradiography. Expression of mRNA was standardized relative to expression of β-actin mRNA.

Steroid binding assays

Osteosarcoma cells were grown to 80% confluence in 75 cm$^2$ tissue culture flasks, trypsinized and then washed twice in serum-free medium. Final cell pellets were resuspended in serum-free medium to give 1 × 10$^7$ cells/ml. Aliquots (200 µl) of cell suspension were then added to glass tubes containing either: (i) increasing concentrations of [3H]dexamethasone ([3H]DEXA) (0·1–20 nM, specific activity 44 Ci/mmol, Amersham); (ii) increasing concentrations of [3H]DEXA (0·1–20 nM)+200-fold excess of cold DEXA; (iii) increasing concentrations of [3H]aldosterone ([3H]ALDO) (0·1–10 nM, specific activity 80 Ci/mmol, Amersham)+200-fold excess of RU486 (Hoechst Marion Roussel, Kansas City, USA); or (iv) increasing concentrations of [3H]ALDO+200-fold excess of cold ALDO+200-fold excess of RU486. Cells were incubated with radiolabelled steroids for 1 h at 37 °C, washed three times with 1 ml cold PBS and the final cell pellet resuspended in 200 µl cold PBS+500 µl cold absolute ethanol. Bound radioactivity was analysed by scintillation counting. Assays for Scatchard plots were performed in triplicate and repeated at least twice.

Results

RT-PCR studies indicated that all four human osteosarcoma cells expressed mRNA for 11β-HSD 2, but not 11β-HSD 1 (Fig. 1A). Subsequent investigation of 11β-HSD activity using whole cell assays is shown in Fig. 1B. Using cortisone as substrate at concentrations near to the $K_m$ value for 11β-HSD 1 (0·3 µM), none of the osteosarcoma cells demonstrated reductase activity (i.e. conversion of cortisone to cortisol). In contrast, all four cell lines were able to convert cortisol to cortisone, indicating dehydrogenase activity, with the highest level of 11β-HSD activity being observed in MG-63 cells. Kinetic analysis of cortisol to cortisone conversion in whole cell preparations of MG-63 indicated the presence of high-affinity dehydrogenase activity (apparent $K_m$ for cortisol=71 nM) (Fig. 2A and B), suggesting 11β-HSD 2 expression. Conversion of cortisol to cortisone at this concentration was linear for incubation periods up to 12 h (data not shown), and no metabolism of cortisol to cortisone, or cortisone to cortisol, was observed in the absence of cells. To confirm that this cortisol to cortisone conversion was mediated by 11β-HSD 2, lysate studies were carried out on MG-63 cells (Fig. 2C), which demonstrated dehydrogenase activity which was enhanced following addition of NAD but not NADP (Fig. 2C). No metabolism of cortisone to cortisol was observed in MG-63 homogenates in the presence of NADH or NADPH (data not shown).
Further studies showed that mRNA for both MR and GR was present in all four human osteosarcoma cell lines (Fig. 3A). This was confirmed using Northern blots probed with labelled cDNAs derived from the PCR reactions (Fig. 3B). The presence of GR and MR protein was assessed by binding studies using $^3$H[DEXA and $^3$H]ALDO. None of the cells showed any specific binding of $^3$H[ALDO (data not shown). The concentration range used was 0.1–20 nM and all assays were carried out in the presence of RU486 to abolish low-affinity binding to GR. In contrast, all of the OB cell lines showed specific binding for $^3$H[DEXA, but Scatchard analysis of binding data (Fig. 4) showed that MG-63 had the highest $^3$H[DEXA-binding capacity (27 000 GR/cell). Binding affinity values ($K_a$) were similar for all four cell lines, being of the order of 10 nM.

Further analysis of 11β-HSD expression was carried out using primary cultures of human OB cells (Fig. 5). RT-PCR determination of 11β-HSD isozyme mRNA expression revealed the presence of a weak band corresponding to 11β-HSD 2, with 11β-HSD 1 being more strongly expressed (Fig. 5A). Parallel assessment of corticosteroid receptor transcripts in primary OB cells showed the presence of mRNA for GR but not MR (Fig. 5A). Activity studies using whole cell OB preparations showed that the predominant activity was still inactivation of cortisol to cortisone (Fig. 5B). However, in contrast to the osteosarcoma cell studies, fractional conversion of cortisol to cortisone was greater at higher concentrations of cortisol (500 nM) in keeping with low-affinity dehydrogenase activity. Kinetic analyses of dose-responsive changes in cortisol to cortisone conversion indicated an apparent $K_m$ of 1.5 µM (data not shown). When 250 nM cortisone was used as substrate, conversion of cortisone to cortisol was observed, but this was always at least ten times lower than cortisol to cortisone conversion.

**Discussion**

Skeletal status is tightly controlled by systemic and local factors, which influence formation and resorption of bone. In particular, abnormal hormonal control of bone remodelling is a central feature of most common bone disorders. For women, post-menopausal loss of circulating oestradiol levels is a major factor in the pathogenesis of osteoporosis. For men and women, osteoporosis can also occur as a result of continued exposure to excessive doses of glucocorticoids. In both forms of osteoporosis there is a clear link between circulating levels of hormone and the development of bone disease. However, not all post-menopausal women suffer osteoporotic fractures and only 30–50% of patients taking long-term glucocorticoid therapy develop the disease (Lukert & Raisz 1990). As a result of this, several recent studies have examined the role of local steroidogenesis as a mechanism for modulating...
bone function. So far, this has focused on the ability of OBs to generate significant amounts of oestrogens from androgens via the enzyme aromatase (Morishima et al. 1995, Nawata et al. 1995). We have extended these studies of the intracrinology of bone by investigating the role of hydroxysteroid dehydrogenases as pivotal regulators of local steroid concentrations. Specifically, we have assessed two particular groups of enzymes, 17\(^{-}\)HSD types 1–4 (Eyre et al. 1998), and 11\(^{-}\)HSD types 1 and 2. In data presented here we have shown for the first time that OB cells are able to endogenously metabolize glucocorticoids by expression of the enzyme 11\(^{-}\)HSD. Analysis of both osteosarcoma cells and primary human OB cells indicates that the predominant activity is conversion of cortisol to cortisone. However, RT-PCR analyses suggest that this is mainly due to the type 1 isoform in primary OB cells and the type 2 isoform in osteosarcoma cells. As both primary OB and osteosarcoma cells appeared to express GR but not MR our data suggest that 11\(^{-}\)HSD may play an important role in controlling glucocorticoid effects on OBs by modulating the availability of the ligand for GR.

Within the last 5 years, two distinct 11\(^{-}\)HSD iso-enzymes have been cloned and characterized in many mammalian species (Tannin et al. 1991, Albiston et al. 1994, Stewart et al. 1994, White et al. 1997). The type 1 isoform acts predominantly as a low-affinity o xo-reductase generating cortisol from cortisone. The enzyme is expressed in several key glucocorticoid target tissues, including liver, lung, gonad, adipose tissue, decidua, pituitary and other neural tissues (Ricketts et al. 1998b). Several studies have indicated that it serves to modulate access of cortisol or corticosterone to the GR at these sites, independently of prevailing circulating levels (Sakai et al. 1992, Monder et al. 1994, Jamieson et al. 1995, Bujalska et al. 1997a). In contrast 11\(^{-}\)HSD 2 has, until recently, served a more defined role. It has somewhat restricted expression in human adult kidney, colon and salivary gland (Stewart et al. 1994, Whorwood et al. 1995) where it co-localizes with the MR, endorsing clinical data which indicate that this isoenzyme protects the MR from illicit occupancy by glucocorticoids. Inactivation of cortisol to cortisone by 11\(^{-}\)HSD 2 enables ALDO to bind to the MR maintaining normal receptor specificity in vivo (Edwards et al. 1988, Funder et al. 1988).

The exceptions to this ‘compartmentalization’ of 11\(^{-}\)HSD 1 and GR, and 11\(^{-}\)HSD 2 and MR, appear to be fetal tissues and, in this study, bone. 11\(^{-}\)HSD 2 is expressed in high abundance in many human fetal tissues including the placenta (Brown et al. 1993, Stewart et al. 1995). Its function within these tissues remains unclear, but does not appear to relate to MR expression, at least in the placenta (Petrelli et al. 1997). In this study, analysis of activity and mRNA levels in osteosarcoma cells suggested the expression of the type 2 11\(^{-}\)HSD isozyme. No metabolism of cortisone to cortisol was detected even at substrate concentrations approaching \(K_m\) values for
11β-HSD 1 (0·3 µM). Conversion of cortisol to cortisone was quantifiable in both whole cell preparations and in cell lysates, where it was clearly NAD dependent. Furthermore, kinetic analysis of cells with the highest activity (MG-63) revealed an apparent

\[ K_d = 9·5 \text{ nM}, B_{	ext{max}} = 8728 \text{ fmoles/10}^6 \text{ cells} \]  

Figure 4 Scatchard analyses of [3H]DEXA binding to human OBs. 

- MG-63, mean \( K_d = 11·3 \text{ nM}, B_{	ext{max}} = 26 874 \text{ GR/cell} \)  
- TE-85, \( K_d = 9·5 \text{ nM}, B_{	ext{max}} = 8728 \)  
- SaOS-2, \( K_d = 11·3 \text{ nM}, B_{	ext{max}} = 8728 \)  
- Hs913T, \( K_d = 13·4 \text{ nM}, B_{	ext{max}} = 5138 \)  

The OB nature of MG-63, TE-85 and SaOS-2 cells has been well documented in previous reports, with each cell line possessing slightly different characteristics (Rodan et al. 1987, Glover & Gowen 1994). TE-85 and SaOS-2 cells show elevated levels of ALP activity compared with MG-63. However, of the three cell lines only MG-63 showed enhanced OC expression following treatment with 1,25-dihydroxyvitamin D₃ (1,25D₃) even though both SaOS-2 and MG-63 cells have been shown to express similar numbers of receptors for 1,25D₃ (Rodan et al. 1987). SaOS-2 cells appear to represent a more mature OB-like phenotype (similar to the rat osteosarcoma cell line ROS 17/2·8), but the response of these cells to glucocorticoids is relatively modest compared with MG-63 or TE-85 cells (Murray et al. 1987). With these observations in mind it is difficult to describe a direct association between OB differentiation and capacity to metabolize glucocorticoids, particularly as the levels of 11β-HSD 2 and GR expression in TE-85 and SaOS-2 cells were similar to those observed in the fibrosarcoma cell line Hs913T. The only clear correlate for 11β-HSD activity in all four cell lines was GR expression, emphasizing further a possible link between regulation of ligand availability and glucocorticoid signalling in OB cells.

Inactivation of cortisol to cortisone was also observed in primary cultures of OB cells. However, in contrast to the osteosarcoma cells, this appeared to be due to expression of the type 1 isozyme of 11β-HSD. RT-PCR analyses indicated that mRNA levels for 11β-HSD 1 were higher than those observed for the type 2 isoform, which was barely detectable in OB cells. Furthermore, enzyme activity studies demonstrated low-affinity dehydrogenase activity, although relatively little reductase activity was observed. This observation is of interest in view of previous analyses of 11β-HSD 1 activity. Studies using cell lysate/homogenate preparations have highlighted predominant dehydrogenase activity, with the reductase appearing to be unstable in vitro (Jamieson et al. 1995). In contrast, data from intact cells such as rat (Jamieson et al. 1995) and human (Ricketts et al. 1998a) hepatocytes as well as neuronal cells (Rajan et al. 1996) suggest that 11β-HSD is exclusively a reductase, generating cortisol from cortisone. These data are in keeping with studies of the expressed human 11β-HSD 1 cDNA (Stewart et al. 1994). Nevertheless, it is well established that 11β-HSD 1 may act predominantly as a dehydrogenase in intact cells from other tissues, notably testis (Monder et al. 1994), and vascular smooth muscle cells (Walker et al. 1991). It is therefore entirely possible that the predominant direction of 11β-HSD 1 activity within a tissue is determined by the redox potential (NADPH/NADP ratio).

Differences between osteosarcoma and OB cells regarding the predominant 11β-HSD isozyme responsible for this metabolism are likely to be due, primarily, to the heterogeneity of the OB cultures. Approximately 40% of the cells obtained by primary culture showed positive staining for ALP and were clearly OB like. However, the cultures would also include a range of OB differentiation states including some fibroblastic OB precursor cells. Fibroblasts are known to express 11β-HSD 1 (Hammami & Siiteri 1991) and this may account for the relatively high levels of 11β-HSD 1 mRNA detected in the primary cultures of OB cells. The outgrowth of OB cells from bone fragments requires relatively long-term culture. However, by using this method it is possible to isolate relatively mature OB cells, with less well-differentiated cells being removed by enzymatic digestion. In this way we were able to assess 11β-HSD expression and activity without stimulating the cells to differentiate, which is usually achieved by treatment with glucocorticoids (Beresford et al. 1984). The apparent lack of a functionally expressed MR in primary bone cultures and osteosarcoma cells suggests that 11β-HSD in human OB cells acts to modulate GR-mediated responses. These data contrast with those from preliminary studies of MG-63 cells, which suggested
low level expression of MR expression (1500 MR/cell) (Allen et al. 1993). Interestingly, this report also highlighted oxidative conversion of corticosterone to 11-dehydrocorticosterone in MG-63 cells, although the specific isozyme responsible for this was not identified. MR expression has also been demonstrated in rat calvarial OB cells, which showed enhanced proliferation following treatment with ALDO (Agarwal et al. 1996). Using rat osteosarcoma cells we have also been able to demonstrate the presence of mRNA for MR as well as specific ALDO binding. Like human osteosarcoma cells, the apparent level of MR expression in rat osteosarcoma cells was much lower than that observed for GR (unpublished data E Rabbitt, LJ Eyre, R Bland, E Walker, SV Hughes, MS Cooper, PM Stewart & M Hewison).

The complex effects of corticosteroids on skeletal tissue are due to a variety of factors including actions on mineral homeostasis (Russell 1993). Of particular interest are the paradoxical effects of glucocorticoids on OBs. Glucocorticoids stimulate the differentiation of pre-OBs to OBs but also inhibit the function of mature OBs (Canalis 1996). At a molecular level, responses to glucocorticoids will in part be due to the level of GR expression as well as other transcriptional factors associated with glucocorticoid-induced gene regulation. Analysis of other corticosteroid target tissues suggests that the activation of GR and MR is also dependent on the local regulation of ligand availability through the concerted action of the type 1 and 2 isozymes of 11β-HSD. The expression and activity of these enzymes in OBs indicate that local regulation of glucocorticoid availability may also be an important aspect of normal bone function. Further characterization of these enzymes in bone will help to clarify the physiological actions of glucocorticoids on bone turnover, as well as providing new insights into the mechanisms involved in steroid-induced bone disease.

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