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Cloning and expression of the bovine 11β-hydroxysteroid dehydrogenase type-2

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Abstract

The bovine 11β-hydroxysteroid dehydrogenase type 2 enzyme (11β-HSD-2) cDNA was cloned from three overlapping PCR fragments using primers based on the human and ovine 11β-HSD-2 cDNA sequences. Both cDNA ends were obtained by a modified RACE (Rapid Amplification of cDNA Ends) method. The bovine 11β-HSD-2 cDNA is 1878 bp long, excluding the poly(A) tail. It consists of a 5′-untranslated region of 133 bp, an open reading frame of 1215 bp and a 3′-untranslated region of 530 bp. Bovine 11β-HSD-2 cDNA is highly homologous to that of the sheep (92%) and less related to the human (67%), rabbit (65%), rat (52%) and mouse (45%) cDNA. The predicted bovine 11β-HSD-2 protein contains 404 amino acid residues with a calculated mol wt of 43,985. It is homologous to the sheep (98%) and human (88%) protein, and less related to that of the rabbit (76%), rat (80%) and mouse (77%). The cloned 11β-HSD-2 cDNA was transfected into CHOP cells and the enzymatic characteristics determined. The enzyme functions primarily as an oxidase, uses NAD^+ and is more active with corticosterone as a substrate than with cortisol or dexamethasone. It is expressed in high concentrations in kidney, adrenal and colon, and in small concentrations in liver, heart and lung. In conclusion, the 11β-HSD-2 enzyme of cattle is very similar to that of other species in its structure and enzymatic characteristics.

1. Introduction

The mineralocorticoid receptor (MR) has similar affinity for the glucocorticoids corticosterone (B) and cortisol (F) as for the mineralocorticoid aldosterone [1]. Under physiologic conditions in vivo, cortisol and corticosterone have minor antinatriuretic activities although they are 100–1000 times as abundant as aldosterone in plasma. The selectivity of the mineralocorticoid receptor for aldosterone is conferred by the presence of 11β-hydroxysteroid dehydrogenase (11β-HSD) in aldosterone target tissues. This enzyme rapidly converts B and F to the inactive metabolites 11-dehydrocorticosterone (A) and cortisone (E), respectively, before they are exposed to the MR [2,3]. Aldosterone in solution has a 11-18-hemiacetal or 11-18-20 bicyclical acetal structure [4], and is not a substrate for this enzyme.

At two isozymes of 11β-HSD have been cloned and characterized. The type 1 isozyme (11β-HSD-1) is $NADP^+$ dependent, has a high K_m of 1–3 μM for B and F, is bidirectional, functioning primarily as a reductase in vivo and in intact cells, and does not colocalize with the MR in the kidney [5]. The type 2 isozyme (11β-HSD-2) is NAD^+ dependent, unidirectional for the natural steroids (dehydrogenase activity only), has a low K_m of 4–14 nM for B and F, which is relevant to circulating levels of free glucocorticoids, and colocates with the MR in aldosterone target tissues [6]. 11β-HSD-2 has been cloned from sheep

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[7], human [8], rabbit [9], mouse [10] and rat [11] renal cDNA libraries. In situ hybridization studies indicate that 11β-HSD-2 mRNA is expressed in the adrenals and female reproductive system in rats, as well as in classic epithelial aldosterone target tissues [12–14]. The 11β-HSD activity has also been shown to modulate glucocorticoid binding to the glucocorticoid receptor [15,16].

This is particularly crucial in the placenta, where 11β-HSD-2 maintains normal fetal blood levels in the face of elevated maternal glucocorticoids [17,18], and in the hypothalamus, where it modulates access of F and B to the hypothalamo-pituitary-adrenal feedback system [19].

Most physiological manipulations of steroid action are performed in the rat, but the glucocorticoid pattern is different from that in man. The physiologic corticosteroids in the bovine are similar to that of the human. To study the regulation of cortisol and cortisone biosynthesis in the bovine adrenal gland, we first designed a ribonuclease protection assay to study mRNA regulation. This required the availability of the bovine cDNA clone. Isolated bovine 11β-hydroxylase can convert cortisol to cortisone [20], and we wanted to investigate whether this conversion was done by 11-HSDs distributed in the adrenal. We report the cloning, expression and tissue distribution of the 11-HSD-2 cDNA in bovine tissues.

2. Materials and methods

2.1. Materials

Steroids, cofactors and materials for buffers were obtained from Sigma Chemical Company (St Louis, MO). Tritiated steroids [1,2^{3}H]-corticosterone, [1,2^{3}H]-cortisol, [1,2^{3}H]-cortisone and [1,2^{3}H]-dehydrocorticosterone were labeled at American Radiolabeled Company (St. Louis, MO) from 1,2 unsaturated precursors prepared in our laboratory. Modified CHO cells (CHOP cells), a transfected line which exhibits very high transfection efficiency, were kindly provided by Dr. James W. Dennis from the Samuel Lunenfeld Research Institute of Mt. Sinai Hospital of Toronto [21].

2.2. RNA and mRNA isolation

Bovine kidneys were obtained immediately after death at an abattoir, placed immediately in liquid nitrogen and processed the same day. Total RNA was extracted from bovine tissues using the Ultraspec Reagent (Biotecx, Houston, TX). Bovine kidney messenger RNA (mRNA) was isolated using the Micro-Fast Track Kit (Invitrogen, San Diego, CA). Total RNA was also extracted from various tissues for determination of tissue distribution.

2.3. Cloning

The bovine 11β-HSD-2 was cloned and sequenced in three fragments by heterologous PCR and RACE (Rapid Amplification of cDNA Ends). The 550–1198 bp fragment of the cloned bovine 11-HSD-2 cDNA was amplified by PCR using primers from the human 11β-HSD-2 cDNA sequence. For reverse transcription, 5 μg total RNA as heated with 100 pmole of an anchored poly-T primer for 5 min at 70°C, reverse transcribed in incubation buffer containing 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM dNTP, for 60 min at 42°C with 200 U of Superscript II Reverse Transcriptase (Gibco BRL, Gaithersburg, MD) in a 20 μl final volume followed by a 5 min inactivation at 95°C. The PCR reaction was done using the primers based on the human 11-HSD-2 sequence: sense (5′-GACCAAACCAGGAGACATTAGC-3′) and antisense (5′-AGGCAGGTAGTAGTGGATGAAG-3′) with the following cycling parameters: 30 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 68°C and a final extension of 7 min at 68°C. The PCR product was purified and sequenced using the Taq DyeDeoxy Terminator Cycle Sequencing Kit and ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA). The 5′ and 3′ ends of the cDNA were amplified using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA). Briefly, first strand cDNA was synthesized using 1 μg mRNA, the primer (5′-TTCTAGAATTCAGCGGCCGC(T)30 (G/A/C)N-3′) and MMLV Reverse Transcriptase. Second strand cDNA was synthesized with an enzyme cocktail of RNase H, *E. coli* DNA polymerase I and *E. coli* DNA ligase, followed by a T4 DNA polymerase treatment to generate blunt ends. Finally an adapter (5′-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT-3′) was ligated with T-4 DNA ligase. The first PCR reaction for 5′ ends was done with the ligated cDNA, the specific primer b-A-1 and the adapter primer AP-1 (5′-CCATCCTAATACGACTCACTATAGGGC-3′), followed by a nested PCR reaction with the specific primer b-A-2 and the adapter primer AP-2 (5′-ACTCACTATAGGGCTCGAGCGGC-3′). Cycling conditions for both PCR and nested PCR reactions were 1 min at 95°C, 5 cycles of 30 s at 95°C and 4 min at 72°C, 5 cycles of 30 s at 95°C and 4 min at 70°C, 25 cycles of 20 s at 95°C and 4 min at 68°C. The nested PCR product was purified and TA-cloned using the pGEM-T Vector System (Promega, Madison, WI), following the manufacturer's protocol. Clones were analyzed by restriction enzyme digestion using the enzyme mixtures

ApaI/PstI and ApaI/PstI/HindIII. Positive clones were isolated and sequenced as described above.

The first round PCR reaction for 3′ ends was done with the ligated cDNA, the specific primer b-S-1 (5′-TACATCGAGCACTTGAATGGGCAG-3′) and the adapter primer AP-1, and Nested PCR reaction was done with the specific primer b-S-2 (5′-GCCCTGTGGTAGATGCCATCACCG-3′) and the adapter primer AP-2. Cycling conditions were 1 min at 94°C followed by 30 cycles of 30 s at 94°C, 4 min at 68°C for first round PCR, 1 min at 94°C followed, by 25 cycles of 30 s at 94°C and 4 min at 68°C for nested PCR. The nested PCR product was purified and sequenced as described above.

2.4. Construction of the expression plasmid

To obtain a full coding region, a PCR reaction was done using the primers b-S-5 (5′-GCCCTCGAGC-CATGGAAAGCTGGCCCTGGCCGTCG-3′) and b-A-4 (5′-TGTTCTAGATGTGACCTGCTCACTGGG-CAGTGG-3′) carrying the restriction enzyme sites for Xho I and Xba I, respectively. The cycling conditions were: 1 min at 94°C, 30 s at 94°C, 3 min at 68°C, and a final extension of 7 min at 68°C. The PCR product was purified and cut with the restriction enzymes XhoI and Xba. The insert was ligated into the expression plasmid pCI/Neo cut with the same restriction enzymes (Promega). Plasmids of two positive clones were purified and used for transient transfection.

2.5. Transient transfection and enzymatic analysis

CHOP cells were seeded in twelve-well plates and grown to 50–80% confluence in Ham-F-10 medium containing 10% Fetal Bovine Serum. Recombinant plasmid (0.5 μg) or pClNeo plasmid as control, and 3 μl LipofectAMINE (Gibco-BRL) in 200 μl serum-free medium were incubated at room temperature for 30 min, followed by the addition of 200 μl serum-free medium. The DNA-LipofectAMINE complex was then added to the cells, which were incubated at 37°C. After 6 h, 400 μl medium containing 20% serum was added to each well. Medium was then changed after 16 h and the cells were allowed to grow for an additional 24 h. To determine the catalytic activity of the bovine 11β-HSD-2, transfected cells in 12-well plates were incubated with 1 ml serum-free medium containing about 200,000 dpm ^{3}H-labeled F or B (6–7 nM) for 2 h at 37°C. The medium was collected and extracted with 5 ml dichloromethane. Ten micrograms of corresponding unlabelled steroid standards were added to the extracts, and the extracts evaporated. The samples were dissolved in 50 μl dichloromethane, spotted onto TLC plates, and developed in dichloromethane:acetone (7:3, v/v) for F and E, and dichloromethane:acetone (82:18, v/v) for B:A or chloroform:acetone (75:25, v/v) for dexamethasone/11-dehydrodexamethasone. The TLC plates were analyzed with a Bioscan Scanner System.

For the reverse reaction and inhibition assays, cells grown in 75 cm^2 flasks were transfected, trypsinized and resuspended in serum free medium. 50,000 Cells were incubated in 0.5 ml medium with 200,000 dpm of ^{3}H-labeled substrate for 2 h at 37°C. The supernatant was processed as described above. For inhibition experiments the assays were done using ^{3}H-cortisol as substrate and increasing concentrations of inhibitors. To determine the cofactor dependence and the K_m, the transfected cells were scraped, homogenized and sonicated briefly in reaction buffer (50 mM Tris–HCl, pH 8.0 and 1 mM $MgCl_2$). The protein concentration of the homogenate was determined by the BCA assay (Pierce Chemical Co., Rockford, IL). The homogenate was mixed with an equal volume of glycerol and kept at −70°C for several weeks without loss of enzymatic activity. To determine the cofactor dependence, 5 μg homogenate protein, 0.5 mM each of NAD^+ or $NADP^+$, and about 200,000 dpm cortisol, corticosterone or dexamethasone were incubated in 500 μl buffer for 15 min at 37°C. To determine the K_m, 2–100 nM labeled substrates were incubated with 0.5 μg homogenate protein and 0.5 mM NAD^+ in 500 μl buffer, at 37°C for 15 min. Product formation was analyzed by TLC as described above, bands were visualized with UV light, scraped from the plate, eluted from the silica with 0.5 ml isopropanol, and separated from the silica by centrifugation. The isopropanol solution was decanted into vials for scintillation counting. All assays were performed in duplicate. Tritiated cortisone, 11-dehydrocorticosterone and 11-dehydrodexamethasone were prepared by incubation of their respective 11-hydroxylated derivatives with 293 cells stably transfected with the human 11β-HSD-2, followed by solid reverse phase extraction of the incubation media and TLC purification.

3. Results and discussion

3.1. Isolation of bovine 11β-HSD-2 cDNA

The bovine 11β-HSD-2 cDNA sequence was obtained by cloning 3 overlapping PCR fragments. Internal fragments were obtained by PCR reaction using primers based on the human and ovine cDNA sequences. Both cDNA ends were obtained by a modified RACE method. The bovine 11β-HSD-2 cDNA is 1878 bp long, not including the poly(A) tail (Fig. 1). It consists of a 5′-untranslated region of 133 bp, an open reading frame of 1215 bp and a 3′-untranslated region of 530 bp. Bovine 11β-HSD-2 cDNA is highly hom-

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   1  ACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGTCTCCCCTGGCTGGTGTCCTGCTGCACCCCG
  67  CGTCCCAGCCCCGAGTCCCAGTCCCTGCTCTTCAGCCCAGTCCCTGCCCCGCCCCGGCCCGGCCGCC
      Met Glu Ser Trp Pro Trp Pro Ser Gly Gly Ala Trp Leu Leu Val Pro Ala     17
 134  ATG GAA AGC TGG CCC TGG CCG TCG GGC GGC GCC TGG CTG CTC GTG CCG GCC
      Arg Ala Leu Leu Gln Leu Leu Arg Ala Asp Leu Arg Leu Gly Arg Pro Leu     34
 185  CGT GCG CTA CTG CAG TTG CTG CGC GCA GAC CTG CGT CTG GGC CGC CCG CTG
      Leu Ala Ala Leu Ala Leu Leu Ala Ala Leu Asp Trp Leu Cys Gln Arg Leu     51
 236  TTG GCG GCG CTG GCG CTG CTG GCC GCG CTC GAC TGG CTG TGC CAG CGC CTG
      Leu Pro Pro Leu Ala Ala Leu Ala Val Leu Ala Ala Thr Gly Trp Ile Val     68
 287  CTA CCC CCG CTG GCC GCA CTT GCC GTG TTG GCC GCC ACC GGC TGG ATC GTG
      Leu Ser Arg Leu Ala Arg Pro Gln Arg Leu Pro Val Ala Thr Arg Ala Val     85
 338  TTG TCC CGC CTG GCG CGC CCG CAG CGC CTG CCC GTG GCG ACT CGC GCG GTG
      Leu Ile Thr Gly Cys Asp Ser Gly Phe Gly Asn Ala Thr Ala Lys Lys Leu    102
 389  CTC ATC ACC GGC TGT GAC TCT GGT TTT GGC AAC GCG ACG GCC AAG AAG CTT
      Asp Thr Met Gly Phe Thr Val Leu Ala Thr Val Leu Asp Leu Asn Ser Pro    119
 440  GAC ACC ATG GGC TTC ACA GTG TTG GCG ACC GTG TTG GAT CTG AAT AGC CCT
      Gly Ala Leu Glu Leu Arg Ala Cys Cys Ser Ser Arg Leu Lys Leu Leu Gln    136
 491  GGG GCC CTA GAG CTG CGT GCC TGC TGT TCT TCT CGT CTG AAG CTG CTA CAG
      Met Asp Leu Thr Lys Pro Gly Asp Ile Ser Arg Val Leu Glu Phe Thr Lys    153
 542  ATG GAC CTG ACC AAG CCA GGA GAC ATT AGC CGT GTG TTG GAG TTC ACC AAG
      Val His Thr Pro Ser Thr Gly Leu Trp Gly Leu Val Asn Asn Ala Gly Gln    170
 593  GTC CAC ACG CCA AGC ACA GGT CTG TGG GGC CTG GTC AAC AAC GCG GGC CAG
      Asn Ile Phe Val Ala Asp Ala Glu Leu Cys Pro Val Ala Thr Phe Arg Thr    187
 644  AAC ATC TTT GTG GCG GAT GCA GAG CTG TGT CCA GTG GCC ACT TTC CGC ACC
      Cys Met Glu Val Asn Phe Phe Gly Ala Leu Glu Met Thr Lys Gly Leu Leu    204
 695  TGC ATG GAG GTG AAC TTC TTT GGT GCA CTA GAG ATG ACC AAA GGC CTC TTG
      Pro Leu Leu Arg Arg Ser Ser Gly Arg Ile Val Thr Val Ser Ser Pro Ala    221
 746  CCA CTG CTG CGT CGT TCA AGT GGT CGA ATT GTG ACC GTG AGC AGC CCA GCA
      Gly Asp Met Pro Phe Pro Cys Leu Ala Ala Tyr Gly Thr Ser Lys Ala Ala    238
 797  GGA GAC ATG CCA TTT CCA TGC TTA GCT GCC TAT GGG ACC TCC AAA GCA GCC
      Leu Ala Leu Leu Met Gly Asn Phe Ser Cys Glu Leu Leu Pro Trp Gly Val    255
 848  TTG GCG TTG CTC ATG GGC AAT TTT AGC TGT GAA CTT CTG CCC TGG GGT GTC
      Lys Val Ser Ile Ile Gln Pro Ala Cys Phe Lys Thr Glu Ser Val Lys Asp    272
 899  AAG GTC AGC ATC ATC CAG CCT GCC TGC TTC AAG ACA GAG TCA GTG AAG GAC
      Val His Gln Trp Glu Glu Arg Lys Gln Gln Leu Leu Ala Thr Leu Pro Gln    289
 950  GTG CAC CAA TGG GAA GAG CGC AAG CAG CAG CTG CTG GCC ACC CTG CCT CAA
      Glu Leu Leu Gln Ala Tyr Gly Glu Asp Tyr Ile Glu His Leu Asn Gly Gln    306
1001  GAG CTG CTG CAG GCC TAT GGT GAG GAC TAC ATC GAG CAC TTG AAT GGG CAG
      Phe Leu His Ser Leu Ser Gln Ala Leu Pro Asp Leu Ser Pro Val Val Asp    323
1052  TTC CTG CAC TCT CTG AGC CAG GCC CTG CCA GAC CTC AGC CCT GTG GTA GAT
      Ala Ile Thr Asp Ala Leu Leu Ala Ala Gln Pro Leu Arg Arg Tyr Tyr Pro    340
1103  GCC ATC ACC GAT GCG CTG CTG GCG GCC CAG CCA CTC CGC CGC TAT TAC CCA
      Gly His Gly Leu Gly Leu Ile Tyr Phe Ile His Tyr Tyr Leu Pro Glu Gly    357
1154  GGT CAT GGC CTG GGG CTC ATA TAC TTC ATC CAC TAC TAC CTG CCC GAG GGT
      Leu Arg Gln Arg Phe Leu Gln Ser Phe Phe Ile Ser Pro Tyr Val Pro Arg    374
1205  CTG CGG CAG CGT TTC CTG CAG TCC TTC TTC ATC AGT CCC TAT GTG CCG AGA
      Ala Leu Gln Ala Gly Gln Pro Gly Leu Thr Ser Ala Arg Asp Ile Ala Gln    391
1256  GCA CTA CAG GCT GGC CAG CCT GGC CTT ACC TCT GCC CGG GAC ATA GCC CAG
      Asp Gln Gly Pro Arg Pro Asp Pro Ser Pro Thr Ala Gln Stop               404
1307  GAC CAA GGC CCT AGA CCG GAT CCT TCT CCC ACT GCC CAG TGA GCAGGTCACAC
1360  GTAGAGCAGCTCCAGCAGAGGAGGTTCCTTGTGCCCTTGCTCCTCCTCCAGGTATTCTGACCCCCAA
1427  GGTCTGCCCTAGAGCCTGGCCCAAAGGACCCCACCCCATGCACTGCCGATGCCACAGGCCAGGCCTG
1494  GTGAGGTGAAGGCTTTCCAGTGAACCTCTGGGCCTCTCCTGCTTCATGAGCCCAAACAGACCCTCCT
1561  GGGCACAAGGCTCCACCATGCAGCTTGCAGAACCCAGCTGGATGGGGAGTTTAGTTCAAGGCTTAAC
1628  TAGAGCCTTAGCCAGGATCCTACAGACAGTGCCTCTGCAAACTAAGGCGAGATTAGGTAGGTTGGGG
1695  ACCCCCTCAGGATTATTTCTTGGCACCAGTGCCTCAGTGCTGTGATTTGAAGGGTGAACCCTGTTTC
1762  TTGACTGGCTCAAGGATTAGGGCCCTGACCACCCACCCCAAGCCCCCCAAGGCACAGGGAGGCTACA
1829  TACTCACCTTATTGCCACTTTTTTAATAAAGACAAATTTTTATTTCTCCT
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Fig. 1. Nucleotide and aminoacid sequence of the bovine 11β-hydroxysteroid dehydrogenase. The cofactor site is underline and the putative active site is double underlined.

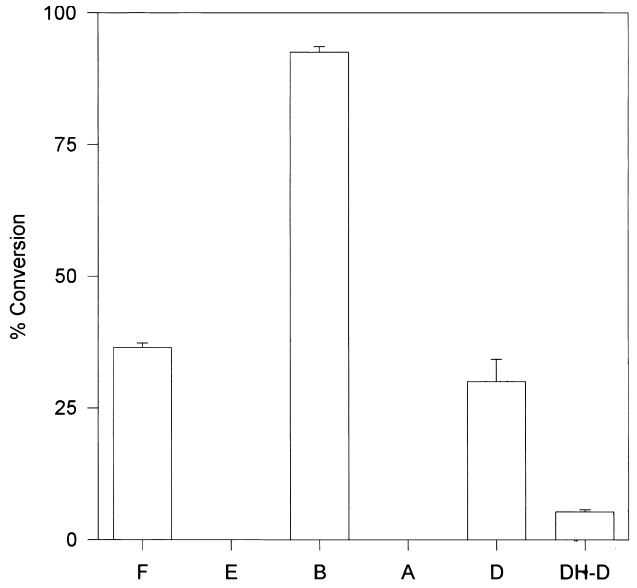

Fig. 2. Percentage conversion of ^{3}H-cortisol (F), ^{3}H-corticosterone (B), ^{3}H-11-dehydrocorticosterone, ^{3}H-dexamethasone and ^{3}H-11-dehydrodexamethasone in CHOP cells transfected with a bovine 11-HSD-2 cDNA (pClNeo-b11-HSD-2) incubated for 2 h at 37°C. Results are mean ± SD.

ologous to the sheep (92%), and less related to the human (67%), rabbit (65%), rat (52%) and mouse (45%). The predicted bovine 11β-HSD-2 protein contains 404 amino acid residues with a calculated mol wt of 43,985. It is homologous to the sheep (98%), human (88%) and less related to the rabbit (76%), rat (80%) and mouse (77%).

3.2. Characteristics of bovine 11β-HSD-2 enzyme

Enzymatic activity was tested in transfected CHOP cells. The direction of the reaction was tested by incubating transfected CHOP cells with cortisol, corticosterone, dexamethasone and their respective 11-oxo derivatives. Bovine 11β-HSD-2 converted 36.5% of F to E, 92.5% of B to A and 29.9% of dexamethasone to 11-dehydrodexamethasone (DH-D). There was no detectable conversion of E or A to their reduced derivatives, and only DH-D was slightly converted to D (5.3%) (Fig. 2). Bovine 11β-HSD-2 enzyme expressed in CHOP cells functions essentially as a steroid dehydrogenase. Several inhibitors were tested using transfected cells incubated with cortisol as substrate (Fig. 3). The most potent inhibitors were carbenoxolone, glycyrrethinic acid and 11α-OH-progesterone with EC50 of 4.88 ± 6.37, 21.56 ± 3.12 and 33.98 ± 3.57 nM, respectively. 11β-OH-Progesterone was more than ten times less potent than 11α-OH-progesterone, with an EC50 of 448.7 ± 66.01 nM.

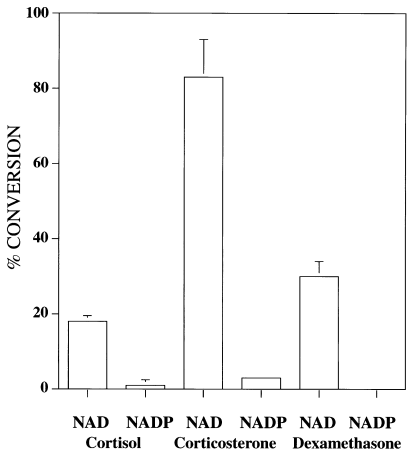

Fig. 4. Cofactor dependence of bovine 11β-HSD-2. Five micrograms of protein homogenates of 11β-HSD-2 transfected CHOP cells were incubated with ^{3}H-cortisol (F), ^{3}H-corticosterone (B) or ^{3}H-dexamethasone (D) for 15 min at 37°C in the presence of 0.5 mM NAD^{+} or $NADP^{+}$. Homogenates without addition of cofactor served as controls. Results are mean ± SD.

3.3. Homogenates

To study the cofactor dependence, homogenates of

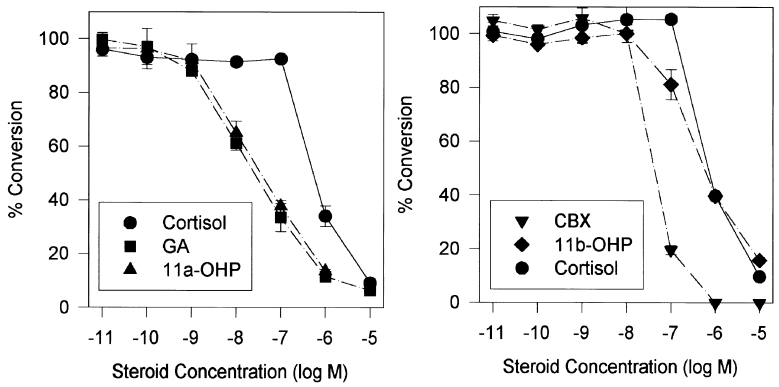

Fig. 3. Inhibition of bovine 11β-HSD-2 in intact transfected CHOP cells by steroids and licorice derivatives. Cells were incubated with increasing concentrations of various compounds and the conversion of ^{3}H-cortisol to ^{3}H-cortisone measured by TLC. Results are presented as percentage of control conversion (±SD).

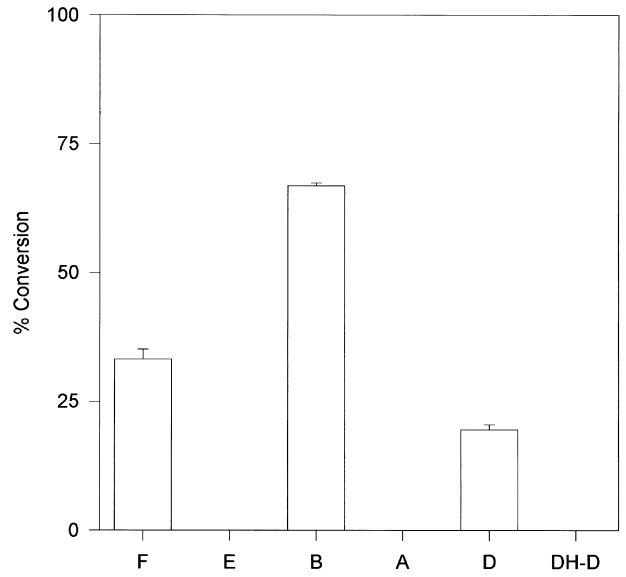

Fig. 5. Determination of the dehydrogenase and reductase activity in homogenates of transfected CHOP cells with bovine 11β-HSD-2. Eight micrograms of protein homogenates were incubated with cortisol (F), cortisone (E), corticosterone (B), 11-dehydrocorticosterone (A), dexamethasone (D) or 11-dehydrodexamethasone (DH-D) for 15 min at 37°C. Results are mean ± SD.

transfected cells were incubated with substrates and NAD^+ or $NADP^+$. Five and 20 μg of protein converted 16.4% and 69.5% of F to E after 15 min incubation with NAD^+; incubation with $NADP^+$ was ineffective (Fig. 4). Five micrograms of bovine 11β-HSD-2 also transformed 84.1% B to A and 30.1% Dexamethasone to 11-dehydrodexamethasone (Fig. 4) with NAD^+ as cofactor. Only corticosterone was slightly oxidized to A using $NADP^+$ as cofactor with a 2.0% conversion compared to 0.7% in the parallel incubation without the addition of either cofactor. 11β-HSD2 reversibility was studied in homogenates by incubating with the 11-oxo derivatives: A, E or 11-dehydrodexamethasone with NAD^+ or NADH as cofactor (Fig. 5). There was no detectable conversion of E, A or DH-D to their respective reduced derivatives. Several inhibitors were tested in homogenates incubated with cortisol as substrate. The most potent inhibitors were 11β-OH-progesterone and 11α-OH-progesterone with EC50s of 1.71 ± 0.65 and 2.41 ± 0.52 nM, respectively; followed by glycyrrethinic acid and carbenoxolone with EC50s of 67.4 ± 9.3 and 96.2 ± 28.7 nM, respectively.

The apparent K_m for the bovine 11β-HSD-2 in homogenates was 45.6 nM, 5.5 nM and 71.8 nM for cortisol, corticosterone and dexamethasone, respectively. V_{max} values were 1595, 474 and 541 pmol/mg·protein·15 min for cortisol, corticosterone and dexamethasone, respectively.

Tissue distribution of bovine 11β-HSD-2: RT-PCR analysis showed that bovine 11β-HSD-2 mRNA is highly expressed in the kidney colon and adrenal gland. Lung and liver contain a smaller amount of message and none was detectable in heart (Fig. 6).

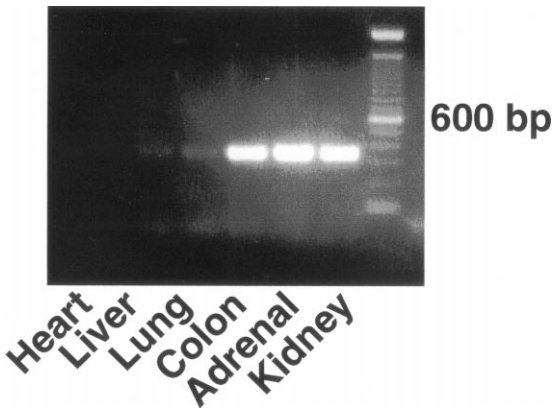

Fig. 6. RT-PCR for the 11β-HSD 2 from RNA prepared from various organs.

The high expression levels of 11β-HSD-2 mRNA in the adrenal gland may explain the observation that a common and abundant metabolite isolated from adrenal incubations in multiple species is cortisone and 11-dehydrocorticosterone [22,23]. The conversion of cortisol into cortisone in bovine adrenal preparations of 11β-hydroxylase has been attributed to the cytochrome P-450 11β-hydroxylase [20], but it is quite likely that the partially purified preparations may have been contaminated by 11-HSD-2.

In conclusion, the kinetic characteristics of the cloned 11-HSD-2 enzyme from the bovine was found to be similar to those found in other species with a higher affinity for corticosterone than cortisol or dexamethasone. Tissue distribution is also very similar to that in other species, predominating in epithelial organs where it can protect the mineralocorticoid receptor from occupancy by the more abundant glucocorticoid cortisol.

Acknowledgements

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