J. Steroid Biochem. Molec. Biol. Vol. 43, No. 4, pp. 343–349, 1992
Printed in Great Britain

0960-0760/92 $5.00 + 0.00
Pergamon Press Ltd

PURIFICATION AND PROPERTIES OF HUMAN HEPATIC 3α-HYDROXYSTEROID DEHYDROGENASE

RADHA B. IYER,[1] JUDITH M. BINSTOCK,[1,4] IRA S. SCHWARTZ,[2] GARY G. GORDON,[1] BERNARD I. WEINSTEIN[3] and A. LOUIS SOUTHREN[1]*

Departments of [1]Medicine, [2]Biochemistry and Molecular Biology, and [3]Ophthalmology, New York Medical College, Valhalla, New York and [4]Department of Biochemistry, New York College of Podiatric Medicine, New York, NY, U.S.A.

(Received 13 April 1992)

Summary—3α-Hydroxysteroid dehydrogenase (3α-HSD) was purified greater than 500-fold from human liver cytosol. The purification was monitored using 5β-[^{3}H]dihydrocortisol (5β-DHF) as substrate. Electrophoretically homogeneous enzyme was obtained using a procedure that involved ammonium sulfate precipitation and three successive column chromatography steps: DEAE-cellulose, hydroxylapatite and Blue-Sepharose. The enzyme is a monomer since the native molecular weight was found to be 37,000, using a calibrated Sephadex G-75 column, and the denatured subunit molecular weight was determined to be 38,500, by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The enzyme had a pI of 5.6–5.9. The 3-ketosteroids: cortisol, testosterone, progesterone and androstenedione, were not substrates for 3α-HSD indicating that a saturated 4,5 double bond was required for substrate activity. The conformation at the 5 position, however, did not influence substrate activity since 5α- and 5β-DHF and 5α-dihydrotestosterone were all reduced at similar rates. The purified enzyme preferred NADPH to NADH as a cofactor and showed a broad peak of activity in the pH range of 6.8–7.4. The apparent K_m for 5β-DHF was 18 μM. The enzyme was markedly stabilized by 50 mM phosphate buffer containing 10 to 20% glycerol at 4°C. Freezing and thawing of the enzyme resulted in a large loss of activity during early stages of the purification. This is the first report of the purification to homogeneity of 3α-HSD from human tissue.

INTRODUCTION

The enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD) is a major component of mammalian liver. The enzyme represents about 1% of the cytosolic protein in the rat liver [1] and metabolizes steroids [2, 3], bile acids [4], prostaglandins [5] and polycyclic hydrocarbons [6, 7]. Cortisol is the major glucocorticoid in the human and is metabolized in the liver predominately to 3α,5β-tetrahydrocortisol (3α,5β-THF) (also known as urocortisol). This metabolism requires the activity of two enzymes: 4-en-3-oxosteroid 5β-reductase (hereafter abbreviated as 5β-reductase) and 3α-HSD. Glucocorticoids are known to elevate intraocular pressure and 5β-dihydrocortisol (5β-DHF) can potentiate this effect [8]. 3α,5β-THF has been shown to lower intraocular pressure [9, 10]. Thus, the activities of the enzymes that reduce cortisol to 3α,5β-THF may be involved in the endogenous regulation of intraocular pressure. We have previously shown that alterations in the activities of the cortisol metabolizing enzymes may be related to the pathogenesis of primary open angle glaucoma [11, 12], an inherited disease characterized by elevated intraocular pressure. As part of a study into the role of cortisol and its metabolites in the regulation of intraocular pressure, we have purified and characterized 3α-HSD from human liver. With the use of a monoclonal antibody, the 3α-HSD in the liver and the eye were found to be immunologically related [13].

*To whom correspondence should be addressed.

EXPERIMENTAL PROCEDURES

Materials

[1,2-^{3}H]Cortisol (55 Ci/mmol), [7-^{3}H]testosterone (23.3 Ci/mmol), 5α-[1,2-^{3}H]dihydrotestosterone (55 Ci/mmol), [1,2,6,7-^{3}H]progesterone (109 Ci/mmol) and [1,2,6,7-^{3}H]androstenedione (92 Ci/mmol) were obtained from New England Nuclear (Boston, MA). 5α-[1,2-^{3}H]DHF (55 Ci/mmol) and 5β-[1,2-^{3}H]DHF were prepared from tritiated cortisol as described

previously [14]. The purities of the radioactive steroids were found to be >95% by either high performance liquid chromatography [4] or thin layer chromatography analysis [11]. Blue-Sepharose, powdered hydroxylapatite (Bio-Gel HTP) and protein assay kit were purchased from Bio-Rad Labs (Richmond, CA). The following materials were purchased from the indicated sources: Polybuffer Exchanger-94, Polybuffer-74/HCl and Sephadex G-75 (Pharmacia, Piscataway, NJ), pyridine nucleotides, DEAE-cellulose (Whatman DE52, preswollen microgranular form) and all other chemicals were of analytical grade (Sigma Chemicals, St Louis, MO). Rainbow molecular weight markers were obtained from Amersham (Arlington Heights, IL).

Adult human male livers were obtained from Stanford Research Institute (Stanford, CA). The livers had been perfused *in situ*, removed and frozen at −70°C.

Assay for 3α-HSD activity

The activity of 3α-HSD was monitored during the purification using 5β-[^{3}H]DHF as substrate, and the assay was carried out as described previously [14]. One unit of 3α-HSD activity was expressed as 1×10^{-4} nmol of 3α,5β-[^{3}H]THF formed in 30 min at 37°C. Specific activity was expressed as U/mg protein.

The identity of the product was determined by comparison of its retention time on high performance liquid chromatography to known standards as described previously [14]. Protein was determined using the Bio-Rad protein assay kit with bovine serum albumin (BSA) as the standard.

Purification of human liver cytosolic 3α-HSD

Step 1. Preparation of the 40–75% ammonium sulfate fraction—300 g of liver were thawed and homogenized in 600 ml of 50 mM phosphate buffer, 1 mM EDTA and 10% glycerol (pH 7.4) in a Waring Blendor (three 20 s pulses) at 4°C. All subsequent procedures were carried out at 4°C. The homogenate was centrifuged at 10,000 *g* for 15 min and the resulting supernatant was centrifuged at 100,000 *g* for 45 min to yield a clear supernatant, the cytosol. The cytosol was then treated with saturated ammonium sulfate (pH 7.0) to obtain the 40–75% saturation fraction which was dissolved in 10 mM phosphate buffer (pH 7.4) and dialyzed overnight against the same buffer.

Step 2. DEAE-cellulose column chromatography—the dialyzed material was applied to a DEAE-cellulose column (2.5 × 45 cm) equilibrated with 10 mM phosphate buffer (pH 7.4). The column was washed with equilibration buffer followed by a 1200 ml linear 0–200 mM gradient of NaCl. The 3α-HSD activity which eluted in the region of 60–100 mM NaCl was pooled and dialyzed against 10 mM phosphate buffer (pH 7.4) containing 10% glycerol.

Step 3. Hydroxylapatite chromatography—the dialyzed protein was applied to a hydroxylapatite column (2.5 × 12 cm) equilibrated with 10 mM phosphate buffer (pH 7.4) containing 10% glycerol. The column was developed with a 400 ml linear 10–200 mM gradient of potassium phosphate buffer (pH 7.4) containing 10% glycerol. The fractions containing 3α-HSD activity which eluted in the region of 50–80 mM potassium phosphate were pooled and dialyzed against 10 mM phosphate buffer (pH 7.4) containing 10% glycerol.

Step 4. Blue-Sepharose affinity chromatography—the dialyzed protein was applied to a Blue-Sepharose column (1.5 × 6 cm) equilibrated with 10 mM phosphate buffer (pH 7.4) containing 10% glycerol. The column was washed with this buffer to remove nonabsorbed protein. The pH was then raised to 8.6 and a second batch of nonspecifically bound protein was eluted from the column. The 3α-HSD activity was eluted with 3 mM $NADP^+$ in 10 mM phosphate buffer (pH 8.6) containing 10% glycerol. The active fractions were pooled and dialyzed against 50 mM phosphate buffer pH 7.4 containing 10% glycerol and stored at 4°C.

Determination of pI of the 3α-HSD

The dialyzed enzyme preparation from the hydroxylapatite column was applied to a chromatofocusing column (1 × 38 cm) packed with Polybuffer Exchanger 94, equilibrated with 25 mM Tris–acetate buffer (pH 7.4). The column was developed using 12 bed volumes of Polybuffer 74/HCl with a linear decreasing pH gradient of 7.4 to 5.0.

Determination of K_m and V_{max}

Using nonradioactive 5β-DHF (0–60 μM) as substrate, 3α-HSD activity, K_m and V_{max} were determined spectrophotometrically as described previously [14].

Determination of molecular weight

A column (2.6 × 90 cm) of Sephadex G-75 was equilibrated with 10 mM phosphate buffer (pH 7.4) containing 10% glycerol. The column was calibrated with the following proteins of known molecular weight: BSA (66,000), ovalbumin (46,000), carbonic anhydrase (29,500) and cytochrome *c* (12,500). Concentrated protein (from the chromatofocusing column in the pI region of 5.6–5.9) was applied to the column

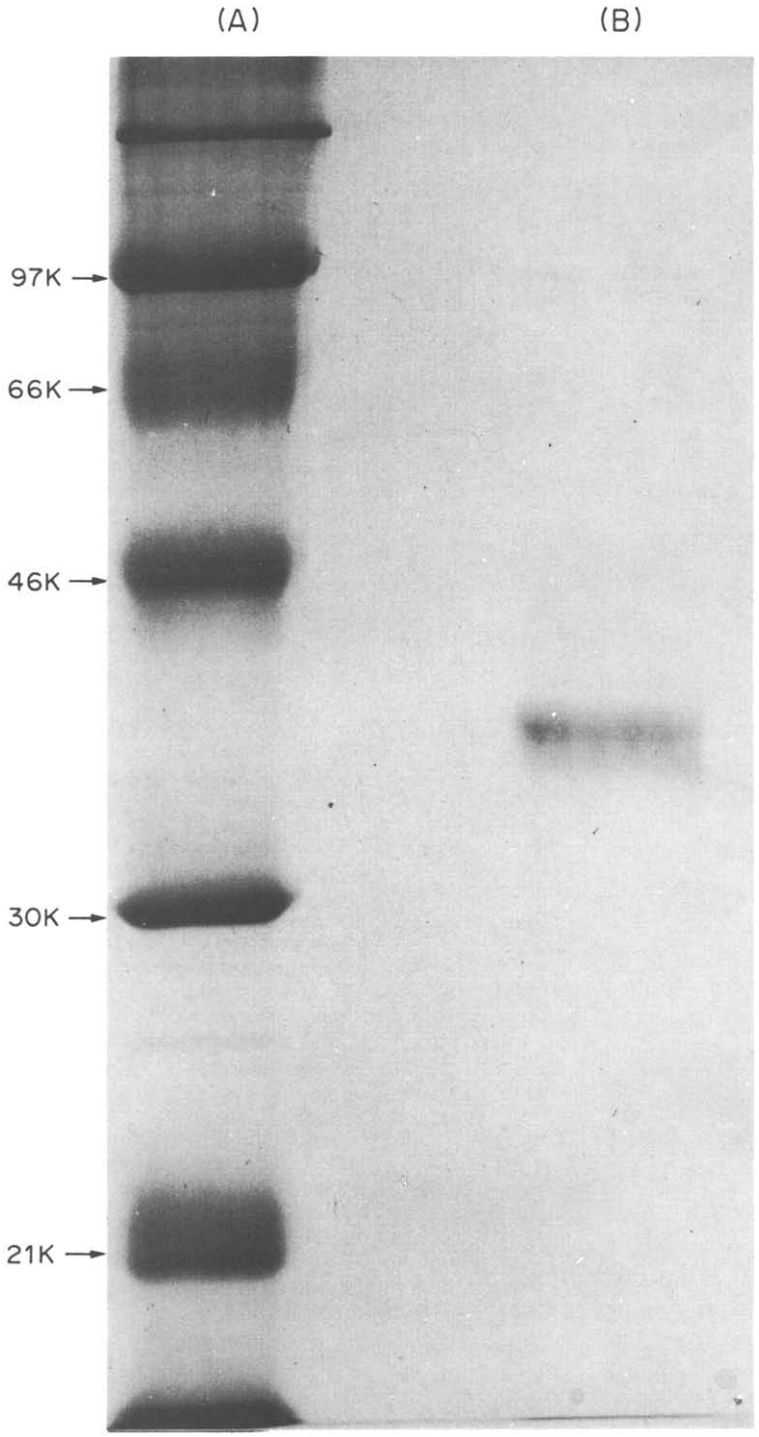

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of human liver 3α-HSD. Lane A, mixture of (1) phosphorylase *b* (M_r = 97,000), (2) BSA (M_r = 66,000), (3) ovalbumin (M_r = 46,000), (4) carbonic anhydrase (M_r = 29,500), and (5) trypsin inhibitor (M_r = 21,000); lane B, the purified 3α-HSD (2 μg).

Table 1. Purification of 3α-HSD from human liver cytosol

Steps	Total protein (mg)	Total activity (U*)	Specific activity (U/mg protein)	Purification (fold)	Yield (%)
Cytosol	6440	103,040	16	1.0	100
$(NH_4)_2SO_4$ 40–70% fraction	2530	45,000	18	1.1	44
DEAE-cellulose	124	190,000	1560	98	184
Hydroxylapatite	16	16,000	1000	62	15
Blue-Sepharose	0.8	6600	8250	516	6

*1 U of activity = 1×10^{-4} nmol of 3α,5β-[^{3}H]THF formed at 37°C in 30 min.

and eluted with the equilibration buffer at a flow rate of 15–16 ml/h.

Purified enzyme was analyzed by denaturing gel electrophoresis in sodium dodecyl sulfate, 12% polyacrylamide (0.75 mm thick) slab gels in a discontinuous buffer system [15]. A low molecular weight calibration kit (97,000, 66,000, 46,000, 30,000 and 21,000) was run in a parallel lane. The gels were stained with 0.1% Coomassie Brilliant Blue.

RESULTS

Purification of human liver 3α-HSD

The overall purification of 3α-HSD from the human liver cytosol is shown in Table 1. Following ammonium sulfate fractionation and three successive column chromatographies (DEAE-cellulose, hydroxylapatite and Blue-Sepharose), a homogeneous preparation of 3α-HSD was obtained (Fig. 1). Although the ammonium sulfate fractionation resulted in no purification of 3α-HSD, it was required to concentrate the enzyme. DEAE-cellulose chromatography partially separated the 5β-reductase from 3α-HSD (Fig. 2). The 5β-reductase was assayed by using [^{3}H]cortisol as substrate. The 3α-HSD containing fractions were pooled and used for further purification. Although this pool contained some 5β-reductase activity, it was removed in subsequent steps. The increase in total 3α-HSD activity observed following DEAE-cellulose chromatography was reproducible. This phenomenon may be due to removal of an inhibitor, although further experiments are necessary to determine the mechanism of this activation. Only 10% of the applied 3α-HSD activity was recovered from hydroxylapatite (50–80 mM potassium phosphate buffer) owing to the need to exclude the contaminating 5β-reductase activity (Fig. 3). Final purification was achieved by chromatography on Blue-Sepharose. The purified enzyme was eluted from Blue-Sepharose with 3 mM $NADP^+$. This active fraction represents a >500-fold purification from the cytosol.

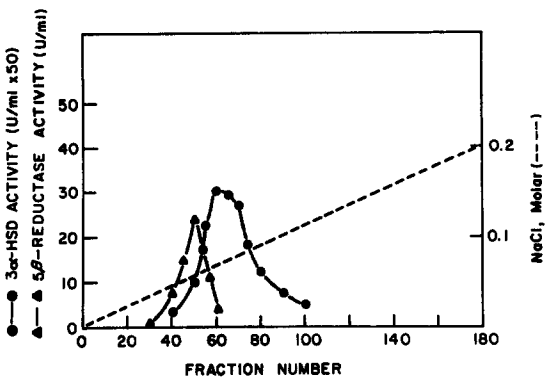

Fig. 2. Elution profile of 3α-HSD from DEAE-cellulose column-chromatography. The dialyzed protein after ammonium sulfate fractionation was applied and eluted as described. The activity of 5β-reductase (▲—▲) was followed with tritiated cortisol as substrate; the activity of 3α-HSD (●—●) was followed with tritiated 5β-DHF as substrate. The NaCl concentration is indicated.

Physical properties of human liver 3α-HSD

The pI of the 3α-HSD, as determined by chromatofocusing, was 5.6–5.9 (Fig. 4). The purified 3α-HSD showed a single band when analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and migrated with an apparent M_r of 38,500 (Fig. 1). Chromatography of native enzyme on a calibrated Sephadex G-75 column showed the 3α-HSD

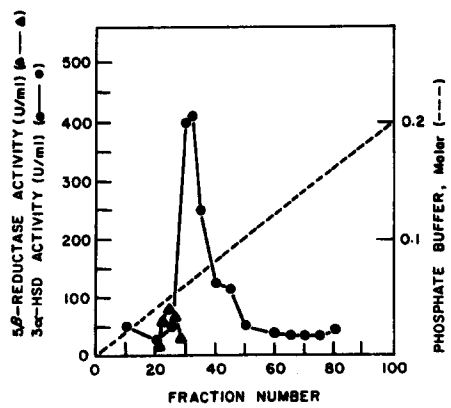

Fig. 3. Separation of 5β-reductase from 3α-HSD on hydroxylapatite column. The active pool of enzyme from (60–100 mM NaCl) DEAE-cellulose was applied to hydroxylapatite and the 5β-reductase (▲—▲) activity and 3α-HSD (●—●) activity was determined as described.

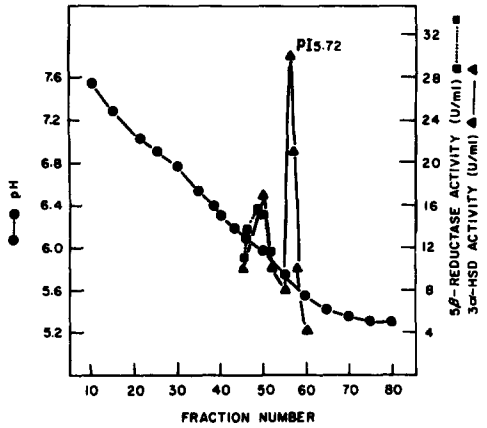

Fig. 4. Chromatofocusing of human liver 3α-HSD. A sample of enzyme from the hydroxylapatite column was applied to this column and eluted with Polybuffer 74, pH 5.0. The pH of the fractions are indicated. Activity was determined as indicated.

activity eluting at 1.3–1.5 void volumes (Fig. 5), corresponding to a M_r of 37,000.

The 3α-HSD activity in the cytosolic fraction is stable in 50 mM phosphate buffer containing 10% glycerol for only 3 days at 4°C. The activity is lost rapidly when the enzyme is frozen during the early stages of the purification even in the presence of 10 or 20% glycerol. By contrast, after the ammonium sulfate precipitation, the activity is stable (>85%) when stored for 2 weeks at 4°C in phosphate–glycerol buffer. Following the hydroxylapatite chromatography the activity was stable in 50 mM phosphate in 20% glycerol at 4°C for 8–12 weeks. The pure enzyme could be stored at −20°C in 50 mM phosphate containing 10% glycerol.

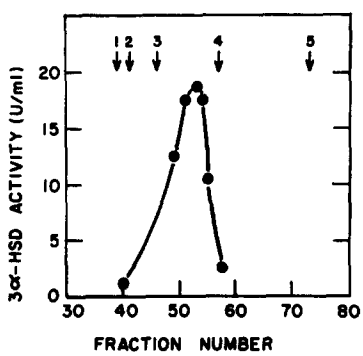

Fig. 5. Sephadex G-75 chromatography of human liver 3α-HSD. The active fractions from chromatofocusing column (pI region of 5.6–5.9) were pooled, concentrated and applied. The column was eluted with the equilibrated buffer. 3α-HSD activity (●—●) was determined as described. The following standards are used: (1) V_o; (2) BSA (66,000); (3) ovalbumin (45,000); (4) carbonic anhydrase (29,500), (5) cytochrome *c* (12,500).

Table 2. Properties of purified 3α-HSD

Products of the reaction	3α, 5β-THF (5β-DHF as substrate) 3α,5α-THF (5α-DHF as substrate)
K_m (5β-DHF as substrate)	18 μM.
V_{max}	4.5 nmol/min/mg protein
pI	5.6–5.9
pH optimum	6.8–7.4
Cofactor requirement	NADPH > NADH
M_r (native)	37,000
M_r (denatured, in SDS)	38,500

Enzymatic properties of human liver 3α-HSD

The purified enzyme had an apparent K_m of 18 μM with 5β-DHF as substrate. The 3α-HSD exhibited a broad pH optimum activity of 6.8–7.4 in phosphate buffer. The reaction proceeded with a 2-fold greater increase in initial velocity with NADPH as cofactor as compared to NADH (Table 2).

The substrate specificity of the 3α-HSD is shown in Table 3. The enzyme was unable to reduce 3-ketosteroids containing a 4,5 double bond (cortisol, testosterone, progesterone or androstenedione). Of the 4,5 reduced 3-ketosteroids, 5α- and 5β-DHF and 5α-dihydrotestosterone were reduced at similar rates. 5β-dihydrotestosterone was not available for testing. When, 5β-DHF was used as substrate, the product of the reaction was identified by high performance liquid chromatography as 3α,5β-DHF whereas when 5α-DHF was used as substrate the product formed was 3α,5α-THF (Table 2).

Inhibition of the 3α-HSD activity by other 3-ketosteroids is shown in Table 4. Dexamethasone and its 21-acetate and hemisuccinate

Table 3. Steroid substrate specificity of purified 3α-HSD from human liver cytosol

Substrates	Relative reaction rates (%)
5β-DHF	100
5α-DHF	81
5α-DHT	98
Cortisol	ND[a]
Testosterone	3
Progesterone	3
Androstenedione	2

Assays were performed using tritiated steroids as described in Experimental Procedures. The rate of each steroid reduction was compared to the initial rate (5×10^{-4} nmol per 30 min) obtained with 5β-[^{3}H]DHF. [a]ND, not detected.

Table 4. Inhibition of 3α-HSD activity[a] by various steroids

Steroid	Concentration (μM)	% Inhibition
Dexamethasone	5	30
Dexamethasone 21-acetate	5	45
Dexamethasone 21-hemisuccinate	5	55
Testosterone	1	75

[a]5β-[^{3}H]DHF was used as substrate.

derivatives all inhibited 3α-HSD activity ($IC_{50} = 5\ \mu M$). Testosterone was a more potent inhibitor ($IC_{50} < 1\ \mu M$).

DISCUSSION

The current paper is the first report of the purification of 3α-HSD from human tissue. Purification of 3α-HSD has been reported from the liver of other species such as rat [16, 17], rabbit [18], mouse [19] and chicken [20], as well as from the rat brain [21], pituitary [22], prostate [23] and epididymis [24]. A homogenous preparation (Fig. 1) was obtained with greater than 500-fold purification (Table 1) from human liver cytosol. The properties of the enzyme are summarized in Table 2 and show similarities to the rat liver enzyme [3, 16]. Both have isoelectric points in the range of 5.6–5.9 with a M_r of 37,000 and use NADPH as the preferred cofactor. Polyacrylamide gel electrophoresis of the purified 3α-HSD in sodium dodecyl sulfate showed a single species of $M_r = 38{,}500$ for the human enzyme. This is similar to that reported for the rat liver cytosolic enzyme [16]. Since this is virtually identical to the M_r determined by gel filtration under native conditions (Fig. 5), the functional enzyme is a monomer.

The human 3α-HSD showed essentially no activity with 3-ketosteroids containing a 4,5 double bond (Table 3). The enzyme reduced both 5α- and 5β-3-ketosteroids at similar rates indicating a lack of conformational specificity at the 5 position. Regardless of the conformation at the 5 position, the enzyme reduced the 3-keto group to a 3α-hydroxy compound.

The purified human 3α-HSD has an apparent K_m for 5β-DHF of 18 μM. This is similar to the value obtained previously for 5α- and 5β-DHF with unfractionated human liver cytosol (18 and 27 μM, respectively) [14]. In rat liver cytosol, using other steroids as substrates, similar values have been reported [3, 16, 25].

It is of interest that testosterone, which is virtually inactive as a substrate, is a potent inhibitor of the enzyme. Previously, we have shown that testosterone and dexamethasone were competitive inhibitors of 3α-HSD in human liver cytosol [14]. High concentrations of dexamethasone were shown to inhibit 3α-HSD noncompetitively in both the rat [16] and mouse liver [19].

Many A-ring reduced products of cortisol [8, 9, 26, 27], progesterone [28–30], testosterone [31] and aldosterone [32–35], which are produced *in vivo* and were previously considered to be inactive, are now known to possess important biological activities. Glucocorticoids have been found to elevate intraocular pressure and 5β-DHF can potentiate this effect [8]. In the rabbit 3α,5β-THF has been shown to lower intraocular pressure [9]. Further, a recent pilot study demonstrated that 3α,5β-THF can also lower intraocular pressure in patients with primary open angle glaucoma [10]. These observations underscore the significance of the cortisol metabolizing enzymes in the regulation of intraocular pressure and possibly in the pathogenesis of primary open angle glaucoma. The importance of the steroid metabolizing enzymes is reflected not only by their ability to metabolize biologically active compounds, but also by their formation of products with opposing activities. This may represent a general mechanism of homeostasis.

REFERENCES

1. Vogel K., Bentley P., Patt K. L. and Oesch F.: Rat liver cytoplasmic dihydrodiol dehydrogenase. *J. Biol. Chem.* **255** (1980) 9621–9625.
2. Gustaffson J. A., Mode A., Norstedt G. and Skett P.: Sex steroid induced changes in hepatic enzymes. *A. Rev. Physiol.* **45** (1983) 51–60.
3. Tomkins G. M.: A mammalian 3α-hydroxysteroid dehydrogenase. *J. Biol. Chem.* **218** (1956) 437–447.
4. Takikawa H., Stolz A., Kuroki S. and Kaplowitz N.: Oxidation and reduction of bile acid precursors by rat hepatic 3α-hydroxysteroid dehydrogenase. *Biochim. Biophys. Acta* **1043** (1990) 153–156.
5. Penning T. M. and Sharp R. B.: Prostaglandin dehydrogenase activity of purified rat liver 3α-hydroxysteroid dehydrogenase. *Biochem. Biophys. Res. Commun.* **148** (1987) 646–653.
6. Penning T. M. and Talalay P.: Inhibition of a major NAD(P)-linked oxidoreductase from rat liver cytosol by steroidal and non-steroidal anti-inflammatory agents and by prostaglandins. *Proc. Natn. Acad. Sci. U.S.A.* **50** (1983) 4504–4508.
7. Smithgall T. E., Harvey R. G. and Penning T. E.: Regio- and stereospecificity of homogeneous 3α-hydroxysteroid dehydrogenase for *trans*-dihydrodiol metabolites of polycyclic aromatic hydrocarbons. *J. Biol. Chem.* **261** (1986) 6184–6191.
8. Southren A. L., Gordon G. G., l'Hommedieu D., Ravikumar S., Dunn M. W. and Weinstein B. I.: 5β-Dihydrocortisol: possible mediator of the ocular hypertension in glaucoma. *Invest. Ophthalmol. Vis. Sci.* **26** (1985) 393–395.
9. Southren A. L., l'Hommedieu D., Gordon G. G. and Weinstein B. I.: Intraocular hypotensive effect of a topically applied cortisol metabolite: 3α,5β-tetrahydrocortisol. *Invest. Ophthalmol. Vis. Sci.* **28** (1987) 901–903.
10. Southren A. L., Wandel T., Gordon G. G. and Weinstein B. I.: Treatment of primary open-angle glaucoma with 3α,5β-tetrahydrocortisol: A new therapeutic modality. *Invest. Ophthalmol. Vis. Sci.* **32** (Suppl) (1991) Abstr. No. 1583 990p.

11. Southren A. L., Gordon G. G., Munnangi P. R., Vittek J., Schwartz J., Monder C., Dunn M. W. and Weinstein B. I.: Altered cortisol metabolism in cells cultured from trabecular meshwork specimens obtained from patients with primary open-angle glaucoma. *Invest. Ophthalmol. Vis. Sci.* **24** (1983) 1413–1417.
12. Weinstein B. I., Munnangi P. R., Gordon G. G. and Southren A. L.: Defects in cortisol metabolising enzyme in primary open angle glaucoma *Invest. Ophthalmol. Vis. Sci.* **26** (1985) 890–893.
13. Iyer R. B. Binstock B. I., Braverman S., Schwartz I. S., Gordon G. G., Weinstein B. I. and Southren A. L.: The formation of the ocular hypotensive metabolite 3α,5β-tetrahydrocortisol by 3α-hydroxysteroid dehydrogenase and its localization in the eye. *Invest. Ophthalmol. Vis. Sci.* **33** (1992) Abstr. No. 2374 1165pp.
14. Iyer R. B., Binstock J. M., Schwartz I. S., Gordon G. G, Weinstein B. I. and Southren A. L.: Human hepatic cortisol reductase activities: enzymatic properties and substrate specificities of cytosolic Δ^4-5β-reductase and dihydrocortisol-3α-oxidoreductase(s). *Steroids* **55** (1990) 495–500.
15. Laemmli U. K.: Cleavage of structural proteins during the assembly of head of bacteriophage T_4. *Nature* **227** (1970) 680–685.
16. Penning T. M., Mukharji I., Barrows S. and Talalay P.: Purification and properties of a 3α-hydroxysteroid dehydrogenase of rat liver cytosol and its inhibition by anti-inflammatory drugs. *Biochem. J.* **222** (1984) 601–611.
17. Ikeda M., Hattori H., Ikeda N., Hayakawa S. and Ohmori S.: Purification and characterization of the multiple form of 3α-hydroxysteroid dehydrogenase in rat liver cytosol. *Hoppe-Seyler's Z. Physiol. Chem.* **365** (1984) 377–391.
18. Worner W. and Oesch F.: Identity of dihydrodiol dehydrogenase and 3α-hydroxysteroid hydrogenase in rat but not in rabbit cytosol. *FEBS Lett.* **170** (1984) 263–267.
19. Hara A., Inoue Y., Nakagawa M., Naganeo F., Sawada H.: Purification and characterization of $NADP^+$-dependent 3α-hydroxysteroid hydrogenase from mouse liver cytosol. *J. Biochem.* **103** (1988) 1027–1034.
20. Morikawa T. and Tamaoki B.: Purification and characterization of 3α-hydroxysteroid dehydrogenase from chicken liver cytosol. *J. Steroid Biochem.* **37** (1990) 569–574.
21. Penning T. M., Sharp R. B. and Krieger N. R.: Purification of 3α-hydroxysteroid dehydrogenase from rat brain cytosol: Inhibition by nonsteroidal anti-inflammatory drugs and progestins. *J. Biol. Chem.* **260** (1985) 15266–15272.
22. Campbell J. S. and Karavolas H. J.: Characterization of the purified pituitary cytosolic NADPH: 5α-dihydroprogesterone 3α-hydroxysteroid oxidoreductase. *J. Steroid Biochem.* **37** (1990) 535–543.
23. Taurog J. D., Moore R. J. and Wilson J. D.: Partial characterization of the cytosol 3α-hydroxysteroid: $NADP^+$ oxidoreductase of rat ventral prostate. *Biochemistry* **14** (1975) 810–817.
24. Hastings C. D. and Hansson V.: Physico-chemical characterization of the NADPH dependant soluble 3α-hydroxysteroid dehydrogenase in the rat epididymis. *Int. J. Androl.* **2** (1979) 263–274.
25. McGuire G. S. Jr, Hollis V. W. Jr and Tomkins G. M.: Some characteristics of the microsomal steroid reductases (5α) of rat liver. *J. Biol. Chem.* **235** (1960) 3112–3117.
26. Marver D. and Edelman I. S.: Dihydrocortisol: a potential mineralocorticoid. *J. Steroid Biochem.* **9** (1978) 1–7.
27. Adam W. R., Funder J. W., Mercer J. and Ulick S.: Amplification of the action of aldosterone by 5α-dihydrocortisol. *Endocrinology* **103** (1978) 465–471.
28. Feder H. H. and Marrone B. L.: Progesterone: its role in the central nervous system as a facilitator and inhibitor of sexual and gonadotrophin release. *Ann. N.Y. Acad. Sci.* **286** (1977) 331–354.
29. Kubli-Garfias C. and Whalen R. E.: Induction of lordosis behavior in female rats by intravenous administration of progestins. *Horm. Behav.* **9** (1977) 380–386.
30. Kubli-Garfias C., Medran-Conde L., Beyer C. and Bondani A.: *In vitro* inhibition of rat uterine contractility induced by 5α- and 5β-progestins. *Steroids* **34** (1979) 609–617.
31. Mainwaring W. I. P.: The mechanism of action of androgens. In *Monographs on Endocrinology* (Edited by F. Gross, M. M. Grumbach, A. Labhart, M. B. Lipsett, T. Mann, L. T. Samuels and J. Zander). Springer-Verlag, NY, Vol. X (1977) pp. 25–29.
32. Sekihara H., Island D. P. and Liddle G. W.: New mineralocorticoid: 5α-dihydroaldosterone and 5α-dihydro-11-deoxy corticosterone. *Endocrinology* **103** (1978) 1450–1452.
33. Kenyon C. J., Brem A. S., McDermott D. J., DeConti G. A., Latif S. A. and Morris D. J.: Antinatriuretic and kaliuritic activities of reduced derivatives of aldosterone. *Endocrinology* **112** (1983) 1852–1856.
34. Morris D. J., Kenyon C. J., Latif S. A., McDermott D. J. and Goodfriend T.: The possible significance of aldosterone metabolites. *Hypertension* [*Suppl*] **5** (1983) I35–I40.
35. Morris D. J. and Brem A. S.: Metabolic derivatives of aldosterone. *Am. J. Physiol.* **252** (1987) F365–F373.