

Oxidative Activity of the Type 2 Isozyme of 17 β -Hydroxysteroid Dehydrogenase (17 β -HSD) Predominates in Human Sebaceous Glands

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Sebum production is regulated by the opposing effects of androgens and estrogens. The intracrine activity of steroid metabolizing enzymes is important in regulating sebum production because these enzymes can convert weak steroids from the serum into potent androgens and estrogens within the sebaceous gland (SG). 17 β -hydroxysteroid dehydrogenase (17 β -HSD) interconverts weak and potent sex steroids via redox reactions. In this regard, it may function as a gatekeeping enzyme regulating the hormonal milieu of the SG. Six isozymes of 17 β -HSD have been identified that differ in their substrate preference and their preference to produce weak or potent sex steroids via oxidation or reduction, respectively. The goals of this study are: (i) to identify which isozyme (s) of 17 β -HSD is active in SG; (ii) to determine if its activity differs in facial skin compared with nonacne-prone skin that may account for the regional differences in sebum production; (iii) to compare the activity of 17 β -HSD in intact glands and in SG homogenates; and (iv) to determine if 13-cis retinoic acid inhibits 17 β -HSD activity. Human SG were assayed for

17 β -HSD activity using estrogens, androgens, and progestins as substrates. Oxidative activity of the type 2 isozyme predominated in all samples tested. Although transcripts for the types 1, 2, 3, and 4 isozymes were detected using reverse transcriptase-polymerase chain reaction, only mRNA for the predominant type 2 isozyme and the type 4 isozyme were detected in northern analysis. Greater reductive activity of 17 β -HSD was noted in SG from facial areas compared with nonacne-prone areas, suggesting an increased net production of potent androgens in facial areas. Oxidation was more predominant over reduction in intact SG compared with homogenized SG, thus supporting the hypothesis that 17 β -HSD protects against the effects of potent androgens *in vivo*. Activity of the type 2 17 β -HSD was not inhibited by 13-cis retinoic acid. In conclusion, SG possess the cellular machinery needed to transcribe the genes for the type 1-4 isozymes of 17 β -HSD. At the protein level, however, oxidative activity of the type 2 isozyme predominates, suggesting that 17 β -HSD isozyme activity may be translationally regulated. **Key words:** acne/sebum/steroid/testosterone. *J Invest Dermatol* 111:390-395, 1998

Sebum production is a key factor in the development of acne. Potent androgens (C-19 steroids) such as testosterone and dihydrotestosterone stimulate sebum production whereas clinical doses of estrogen (C-18 steroids) may inhibit its production (Pochi and Strauss, 1976). The local (or intracrine) metabolism of sex steroids plays a critical role in establishing the balance between androgens and estrogens in the sebaceous gland (SG). At the time of adrenarche, the systemic circulation provides an abundant reservoir of biologically weak steroids including dehydroepiandrosterone sulfate, androstenedione, and estrone. These precursors can be converted into potent C-19 and C-18 steroids within the SG through the action of steroid metabolizing enzymes present in SG (Hay and Hodgins, 1974; Itami and Takayasu, 1981; Simpson *et al*, 1983; Sawaya *et al*, 1988). Because 17 β -hydroxysteroid dehydrogenase (17 β -HSD)

catalyzes both the formation of testosterone and estradiol (via reduction) and the backconversion of these hormones to less active precursors (via oxidation), its activity may regulate the local availability of potent sex steroids in the SG and other endocrine target tissues.

In addition to classical biochemical regulation of steroid metabolizing enzymes (such as pH, availability of substrate and pyridine nucleotide cofactors, etc.), regulation also exists through the tissue specific expression of various isozymes of steroid metabolizing enzymes and even by the action of tissue specific promoters (Simpson *et al*, 1997). Six isozymes of 17 β -HSD have been identified that differ in their preference for substrate (androgens, estrogens, or progestins), pH optima, cofactor specificity, and tissue localization (Luu-The *et al*, 1989; Adamski *et al*, 1995; Andersson and Moghrabi, 1997; Biswas and Russell, 1997). Each isozyme is the product of a unique gene. There is less than 28% sequence homology among the six isozymes. The type 1 isozyme of 17 β -HSD preferentially reduces estrone to estradiol using NADPH as a cofactor. It is the predominant isozyme responsible for estrogen production in the ovary and breast. The type 2 isozyme preferentially oxidizes potent C-19 and C-18 steroids to weaker androgens and estrogens at alkaline pH using NAD as a cofactor. It has been localized to the breast, placenta, ovary, prostate, pancreas, and other tissues. The type 3 isozyme, localized in testis, preferentially reduces androstenedione to testosterone using NADH

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Abbreviations: OX/RED ratio, oxidation/reduction ratio; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; SG, sebaceous gland; 13-cis RA, 13-cis retinoic acid.

or NADPH as a cofactor. The type 4 isozyme oxidizes estradiol to estrone but does not oxidize testosterone, and is a peroxisomal enzyme found in a wide variety of tissues. The type 5 isozyme of 17 β -HSD has been sequenced in mice (Deyashiki *et al*, 1995), and is an aldoketoreductase that preferentially reduces androgens and estrogens using NADPH as a cofactor (Labrie *et al*, 1997). A cDNA encoding a type 6 isozyme was isolated from rat ventral prostate (Biswas and Russell, 1997), and shares 65% sequence identity with the retinol dehydrogenase 1 enzyme. It preferentially oxidizes 3 α -androstenediol to androsterone using NAD⁺ as a cofactor and oxidizes testosterone and estradiol.

In assaying 5 α -reductase activity in human SG, increased back-conversion of testosterone to androstenedione was noted with increasing pH, indicating activity of 17 β -HSD (Thiboutot *et al*, 1995). At the same time, tissue specific expression of 17 β -HSD isozymes was being described. The goals of this study were to identify which isozyme (s) of 17 β -HSD is active in human SG using biochemical parameters, northern analysis, and the reverse transcriptase-polymerase chain reaction (RT-PCR), and to determine if there are differences in its activity in acne-prone regions compared with nonacne-prone regions. In addition, the ratio of oxidative to reductive activity (OX/RED ratio) of 17 β -HSD has been reported to be greater in intact cells compared with homogenized cells (Luu-The *et al*, 1995; Castagnetta *et al*, 1996; Carruba *et al*, 1997). The OX/RED ratio obtained in intact glands may be more representative of the *in vivo* situation. For this reason, the biochemical profile of 17 β -HSD was compared in intact and homogenized SG. Furthermore, it has been hypothesized that 13-cis retinoic acid (13-cis RA), the most potent inhibitor of sebum production, acts in part by inhibiting steroid metabolizing enzymes (Boudou *et al*, 1995). Activity of the rat type 6 isozyme of 17 β -HSD is inhibited by 13-cis RA in transfected human kidney cells (Biswas and Russell, 1997). These data provided a rationale for examining the effects of 13-cis RA on 17 β -HSD activity in SG. Alteration of the activity of the isozymes of steroid metabolizing enzymes such as 17 β -HSD may represent novel strategies in the treatment of hormonally regulated skin diseases such as acne, hirsutism, or male pattern hair loss.

MATERIALS AND METHODS

Skin specimens Samples of normal skin were obtained from routine surgeries performed in the Division of Dermatology at The Pennsylvania State University's Hershey Medical Center under a protocol approved by the Institutional Review Board. Subjects ages ranged from 35 to 85 y of age, with a mean age of 68 y. Samples were transported and SG were dissected as previously described (Thiboutot *et al*, 1995).

Enzyme assays and steroid separation Incubation studies with radiolabeled steroid substrate were performed on homogenized SG and intact SG isolated from facial and nonfacial areas in order to determine the specific activity of 17 β -HSD. Homogenates of SG were prepared and protein content determined as previously described (Thiboutot *et al*, 1995). The incubation cocktail consisted of 10⁶ dpm of radiolabeled steroid substrate ([1,2-³H]testosterone, [1,2,6,7-³H]androstenedione, [6,7-³H]estradiol, [6,7-³H]estrone, [1,2-³H]progesterone, or [1,2-³H]20 α -hydroxyprogesterone; Dupont NEN, Wilmington, DE or Amersham, Arlington Heights, IL) in the presence of 1–10 μ M nonradioactive substrate, 500 μ M NADPH, NADP, NADH, or NAD (Sigma, St. Louis, MO), 1 mM dithiothreitol (Sigma), 40–80 μ g of homogenate protein in a final volume of 0.2 ml of succinic acid/imidazole/diethanolamine buffer with pH adjusted depending on the study being performed. All steroids were purified using thin layer chromatography prior to use. Unless stated otherwise, reactions were performed at pH 7.

In all experiments, each sample was assayed in duplicate and a negative control, consisting of all components of the incubation cocktail except for the SG sample, was assayed under identical conditions. Each reaction was carried out at 37°C for 60 min. The reactions in homogenates were terminated by the addition of 1 ml of cyclohexane/ethyl acetate (70:30, vol/vol) containing nonradioactive carrier steroids. In studies of intact glands, the reactions were terminated by removing the glands from the wells. Samples were extracted twice with chloroform/ethyl acetate (70:30) and the pooled extracts evaporated under nitrogen. The extracts were dissolved in ethyl acetate (androgens and progestins) or ethanol (estrogens) prior to chromatographic separation.

Steroids were separated by thin layer chromatography and enzyme activities were calculated (Thiboutot *et al*, 1995). The solvent system used for separating testosterone from androstenedione and progesterone from 20 α -hydroxyproges-

terone was chloroform/methanol (99:1, vol/vol), whereas estrone and estradiol were separated using chloroform/acetone (9:1, vol/vol).

Assay validation Preliminary experiments to determine the optimal incubation time and linearity of 17 β -HSD activity with SG enzyme protein concentration were performed. SG homogenates from female breast skin were incubated at pH 7 for 10, 60, and 120 min to determine the optimal incubation time for this assay. Androgens were used as substrates. These data were used to determine the time course of the oxidative and reductive activity of 17 β -HSD. Incubation studies with varying quantities of SG homogenate protein (42–340 μ g) were performed in order to demonstrate that the formation of product (androstenedione) was linear with the amount of enzyme used.

Biochemical parameters of 17 β -HSD activity in SG homogenates In order to determine which isozyme(s) of 17 β -HSD predominates in SG, a series of experiments was performed to demonstrate the enzyme's pH optima, cofactor dependence, preference for substrate, and predominant reaction type (oxidation or reduction). SG homogenates from breast tissue were incubated at pH 5–12 in order to demonstrate the pH profile of the oxidative and reductive activity of 17 β -HSD using both C-19 and C-18 steroids as substrates. The dependence of 17 β -HSD activity on the presence of cofactor [500 μ M NAD, NADP, NADH, or NADPH (Sigma)] was demonstrated in incubation studies using C-19 as substrates in homogenized SG from the breast, nose, forehead, and abdomen. Studies to determine the substrate preference of 17 β -HSD were performed in SG homogenates and in intact SG from a variety of anatomic areas.

17 β -HSD activity in intact SG Approximately 70 to 80 SG were freshly isolated from skin from each of seven subjects. For each subject, SG were placed in groups of six into wells of a 24 well cell culture plate for a total of 12 groups of glands for each subject. Duplicate groups of glands were assayed for 17 β -HSD activity with one of the following six substrates: testosterone, androstenedione, estradiol, estrone, progesterone, or 20 α -hydroxyprogesterone (Sigma). Each group of glands was incubated with 10⁶ dpm of radiolabeled substrate (as above) and 1 μ M of nonradioactive substrate in the buffer system described above. No exogenous cofactors were added. Each group of intact SG was homogenized after the incubation and the protein content determined. In all experiments of intact glands, both the oxidative and the reductive activity of 17 β -HSD was determined and the OX/RED ratio was calculated. Comparison was made between the ratio obtained in intact SG and in SG homogenates using a paired *t* test (α = 0.05).

Effects of 13-cis RA on 17 β -HSD activity To determine if 13-cis RA inhibits activity of 17 β -HSD in human SG, enzyme assay solutions from breast SG were supplemented with dilutions of 13-cis RA (Sigma) in ethanol to achieve final concentrations ranging from 0 to 100 μ M. Retinoids were handled under dimmed yellow light. The final concentration of ethanol in each assay was less than 0.5%, a concentration shown not to affect enzyme activity. Testosterone (10 μ M) was used as a substrate and the incubation was carried out for 60 min as above.

Northern analysis Biochemical data suggested that the type 2 isozyme predominated in SG. Northern analysis was performed in order to confirm these data and to determine if messenger RNA from any of the other isozymes could be detected in human SG. Two hundred and fifty SG isolated from eight subjects were pooled, snap frozen in liquid nitrogen, and stored at –80°C. RNA from these glands and from homogenates of control tissues (breast parenchyma, prostate, and placenta) was isolated (Chomczynski Sacchi, 1987). Twenty micrograms of total RNA from each sample were electrophoresed on a 1% agarose gel and transferred to a nylon membrane. A riboprobe complementary to the type 1 17 β -HSD was generated from the full-length cDNA provided by Dr. Van Luu-The (Laval University, Quebec City). Inserts corresponding to the full-length transcripts for the types 2 and 3 17 β -HSD were cut from the pCMV6 plasmids provided by Dr. Stefan Andersson (South-western Texas University, Dallas, TX). These were labeled with ³²P dCTP by random priming (Feinberg and Vogelstein, 1983; Feinberg and Vogelstein, 1984). A probe for the type 4 isozyme of 17 β -HSD was generated by random priming from the cDNA provided by Dr. Jerzy Adamski (GSF National Research Center, Neuherberg, Germany). The nylon membrane was hybridized with probe for 24 h at 42°C, washed under low and high stringency conditions, and exposed to X-ray film (Sambrook *et al*, 1989). After each use, the membrane was stripped of radioactive probe and this procedure was verified by exposure to X-ray film before hybridizing to the next probe. The membrane was hybridized with a radiolabeled probe for 18S RNA to assure equal loading of RNA samples.

Synthetic oligonucleotides The following primers were chosen for each of the four isozymes of 17 β -HSD from published sequences (Luu-The *et al*, 1989; Wu *et al*, 1993; Geissler *et al*, 1994; Adamski *et al*, 1995): type 1 (Genbank

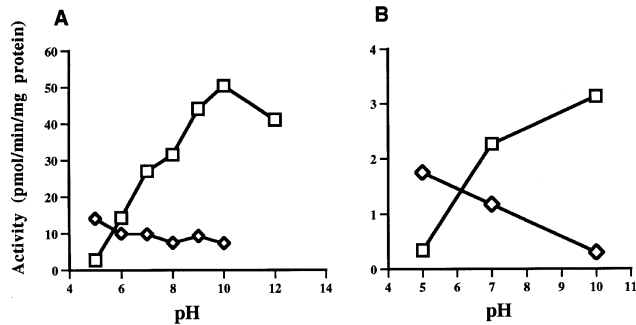


Figure 1. pH profile of 17 β -HSD in homogenates of SG obtained from female breast tissue. (A) Oxidative activity (□) and reductive activity (◇) using androgens as substrates and (B) oxidative (□) and reductive activity (◇) using estrogens as substrates. Values represent the mean of two determinations.

#X13440)FP: 5' CTACCAATACCTCGCCACA; type 1 RP:5'GGTGAAG-TAGCGCAGGGTCG; type 2 (Genbank #L1708) FP: 5'AAGGCTGGCAT-CTTATGGCT; type 2 RP 5'TTCCACATTGTCACCTGGTGCCTGCGAT-ATT; type 3 (Genbank #U05659) FP: 5' ATCCATTGTAACATCACCTC, type 3 RP 5'GGATGATGACTTCTTTTGGCT; type 4 (Genbank #X87176) FP: 5'ATCAGCTTCAGGAATATATG; and type 4 RP 5'CAAGATCTT-CAGGCATAACT. The lengths of the DNA segments to be amplified were 121 base pairs, 151 base pairs, 211 base pairs, and 181 base pairs, respectively, for each of the types 1-4 isozymes.

Reverse transcription and polymerase chain reaction (PCR) Total RNA was isolated from two samples of SG, each pooled from facial areas of 15 subjects (Chomczynski and Sacchi, 1987). First strand cDNA synthesis was performed using a First-Strand cDNA Synthesis kit (Pharmacia, Piscataway, NJ). Briefly, four 1 μ m aliquots of each RNA sample were each diluted in 8 μ l of sterile distilled water, heated to 65°C for 10 min, and cooled on ice. Moloney-mouse-leukemia virus reverse transcriptase 20 u (Pharmacia), 50 pmol of reverse primer, 15 mM dithiothreitol, bovine serum albumin, and dNTP. Samples were incubated at 37°C for 1 h. The polymerase chain reaction was initiated. The reaction product (15 μ l) was then heated to 95°C for 5 min and placed on ice. T_{aq} polymerase 2.5 units (Promega, Madison, WI) and 50 pmol each of forward and reverse primers were added in a final volume of 50 μ l of sterile water. The PCR conditions were set up as follows: 95°C for 5 min, 95°C for 1 min, 58°C for 1 min, 72°C for 1 min. Thirty cycles between steps 2 and 4 were performed. Final extension was performed at 72°C for 10 min. The annealing temperature used for the type 1 primer was 58°C. PCR products were separated by electrophoresis on a 2% agarose gel. Bands were cut from the gel, and DNA was extracted and ligated into a pCR-2.1 vector (Invitrogen, Carson City, CA) containing an ampicillin resistance gene and lacZ α fragment to allow for blue-white screening. Plasmids were transformed into competent cells and colonies were selected based on ampicillin resistance and β -galactosidase activity. Plasmid DNA was prepared from an overnight culture and sequenced for verification using the M-13 reverse primer using an automated DNA sequencer with fluorescent tag (ABI Prism 377, Perkin Elmer, Foster City, CA).

RESULTS

Biochemical parameters of 17 β -HSD in homogenized SG are consistent with activity of the type 2 isozyme Linearity of enzyme activity with time and protein concentration was demonstrated between 10 and 120 min and 40 and 300 μ g protein, respectively (data not shown). The pH profile of the oxidative and reductive activity of 17 β -HSD in homogenized SG from the female breast was determined using both C-19 and C-18 steroids as substrates (Fig 1). With each substrate oxidative activity was sensitive to pH, with optimal activity noted at pH 10. Optimal reductive activity was noted at pH 5.0. For C-19 steroids, reductive activity changed little with pH. The cofactor preference for the oxidative and reductive reactions of 17 β -HSD in SG homogenates using androgens as substrates is demonstrated in Table I. Oxidative activity of 17 β -HSD is greatest with the cofactor NAD in all samples tested. Reductive activity did not show a preference for either NADH or NADPH.

The preference of 17 β -HSD for substrate was demonstrated in homogenized SG from facial areas. Seven homogenates were prepared by pooling \approx 200-300 SG from a particular anatomic area: two homogenates each were prepared from male cheek and male nose and

Table I. Sebaceous glands oxidize androgens using NAD as a cofactor^a

Tissue	Specific activity (pmol per min per mg protein) ^b			
	T \rightarrow androstenedione ^c (oxidation)		androstenedione \rightarrow T + DHT ^c (reduction)	
	NAD	NADP	NADH	NADPH
Breast	16.3 (13.4, 19.3)	1.1 (1.2, 1.0)	7.4 (6.6, 8.2)	7.7 (9.1, 6.3)
Nose	45.8 (45.7, 45.8)	7.7 (7.5, 7.9)	33.3 (32.6, 33.8)	32.8 (34.9, 30.5)
Forehead	14.5 (15.3, 13.7)	0.87 (0.7, 0.9)	6.7 (4.0, 7.2)	4.7 (4.7, 4.7)
Abdomen	55.7 (52.6, 58.8)	14.4 (14.7, 15.9)	21.1 (19.8, 22.3)	18.1 (13.9, 19.1)

^aHomogenates of SG from various areas were assayed for the oxidative and reductive activity of 17 β -HSD using 10 μ M substrate and 500 μ M cofactor.

^bData represent mean (range) of two determinations.

^cT, testosterone; DHT, dihydrotestosterone.

Table II. Homogenized and intact SG prefer to oxidize androgens and estrogens^a

Substrate	Homogenized SG		Intact SG	
	Activity ^b	OX/RED ^c	Activity ^b	OX/RED ^c
Testosterone	10.0 \pm 0.94	1.38 \pm 0.06	0.49 \pm 0.13	3.2 \pm 1.0
Androstenedione	7.37 \pm 0.75	0.14 \pm 0.04		
Estradiol	12.1 \pm 0.98	1.44 \pm 0.11 ^d	1.06 \pm 0.22	7.74 \pm 1.9 ^d
Estrone	8.5 \pm 0.9	0.18 \pm 0.03		

^aSeven samples of sebaceous glands from facial areas described in the text were assayed at pH 7 in homogenized and in intact SG for the oxidative and reductive activity of 17 β -HSD using C-19 and C-18 as substrates (1 μ M). Cofactor (500 μ M) was added in assays of homogenates, but not intact glands.

^bSpecific activity (pmol per min per mg protein). Data expressed are mean \pm SEM of seven determinations.

^cOX/RED ratio represents the mean ratio (\pm SEM) of the specific activity obtained using testosterone or estradiol as a substrate divided by the specific activity using androstenedione or estrone as substrates, respectively. Ratios obtained with each of the seven data sets were used to determine the mean.

^dThe OX/RED ratio obtained in intact glands using estrogens as substrates was significantly greater than the ratio obtained in homogenized glands, $p = 0.03$, paired t test, $\alpha = 0.05$.

one homogenate each from male forehead, female cheek, and female forehead. Each homogenate was assayed in duplicate using C-19, C-18, and C-21 (progestins) steroids as substrates (Table II, C-21 data not shown). Specific activity of 17 β -HSD was greatest using estradiol as a substrate followed by testosterone, estrone, androstenedione, progesterone, and 20 α -hydroxyprogesterone, respectively. The specific activity of 17 β -HSD with C-18 steroids as substrates was \approx 20% higher compared with C-19 steroids. Oxidation was the preferred reaction type in all samples tested. The ratio (mean \pm SEM) of oxidative activity to reductive activity was 1.38 \pm 0.06 with C-19 steroids and 1.44 \pm 0.11 with C-18 steroids.

Northern analysis of SG RNA reveals hybridization to the type 2 probe

Using total SG RNA, a hybridization signal was noted with probe to the type 2 isozyme of 17 β -HSD (1.5 and 2.2 kb transcripts) and the type 4 isozyme (1.4 kb) but not with probes to the types 1 or 3 isozymes (Fig 2). Hybridization signals and transcript sizes in control tissues were consistent with published reports (Luu-The *et al*, 1989; Wu *et al*, 1993; Geissler *et al*, 1994; Adamski *et al*, 1995; Zhang *et al*, 1996). Hybridization to the type 1 probe was noted in prostate, placenta, and breast tissue (2.2 kb transcripts in prostate and breast and 1.3 kb transcripts in all three). Hybridization with the type 2 probe was noted in prostate, placenta, and breast (1.5 kb and 2.2 kb transcripts). The type 3 probe hybridized to RNA in prostate, placenta, and breast (1.0 kb transcripts in prostate and breast and 1.3 kb transcripts in all three) and the type 4 probe hybridized to RNA in

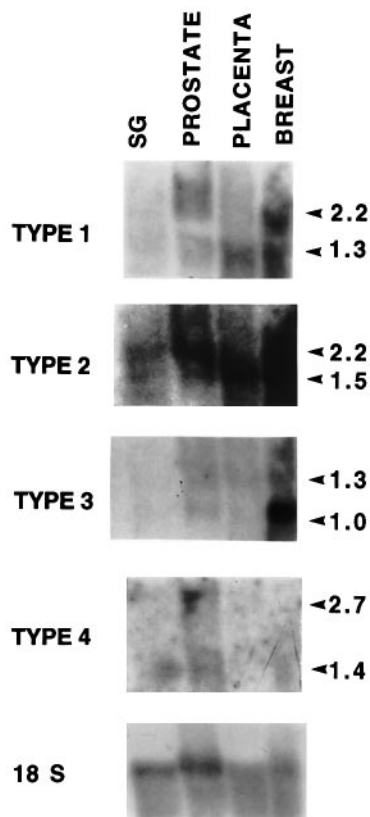


Figure 2. Northern analysis reveals hybridization of the type 2 probe with SG RNA from facial regions. Blot hybridization of human SG, prostate, placental, and breast RNA with 17 β -HSD types 1, 2, 3, and 4 probes. Total RNA (20 μ g) from human SG, prostate, placenta, and breast (lanes 1–4, respectively) were subjected to electrophoresis in the presence of formaldehyde and blotted to a nylon membrane. The membrane was hybridized using high stringency conditions with 32 P-labeled probes (10^6 – 10^7 counts per min per ml) complementary to the entire 17 β -HSD cDNA inserts. The membrane was exposed to X-ray film with two intensifying screens for varying times at -80°C .

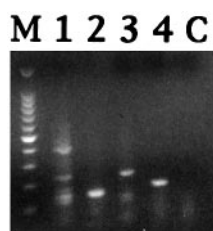


Figure 3. Amplified sequences consistent with types 1, 2, 3, and 4 17 β -HSD are detected in SG by RT-PCR. Total RNA was extracted from microdissected SG. cDNA was synthesized and PCR was performed for each of the four isozymes of 17 β -HSD. SG RNA was run as a negative control in the PCR reaction. M, 100 bp markers; 1, type 1 reaction; 2, type 2 reaction; 3, type 3 reaction; 4, type 4 reaction and C, SG RNA. Expected sizes of amplified sequences based on primer design were 121 bp for type 1, 151 bp for type 2, 211 bp for type 3, and 181 bp for type 4.

prostate (1.4 and 2.7 kb transcripts) and SG as above. Subsequent hybridization of this membrane with 18S RNA indicated that comparable amounts of intact RNA were loaded in each lane.

Types 1, 2, 3, and 4 isozyme transcripts are detected using RT-PCR PCR products from the four reactions from each RNA sample were separated on a 2% agarose gel. Three bands (400 bp, 200 bp, and 121 bp) were noted when primers amplifying the type 1 sequence were used (Fig 3, lane 2). Using primer to the type 2 isozyme, a single distinct band of ≈ 151 bp was noted (Fig 3, lane 3). In the reaction amplifying a type 3 sequence, a distinct band of ≈ 211 bp and a faint band of ≈ 120 bp were noted (Fig 3, lane 4). A 181 bp band was

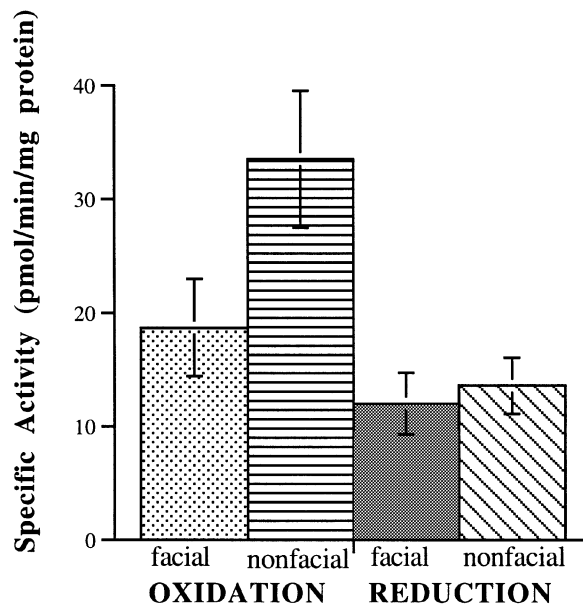


Figure 4. SG from nonacne-prone regions exhibit greater oxidative activity than SG from the face. Oxidative and reductive activities of 17 β -HSD were assayed in 11 homogenates from facial regions and six homogenates from nonfacial (nonacne-prone) regions using androgens as substrates at pH 7, 10 μ M substrate and 500 μ M NAD $^+$ or NADH. Data represent the mean (\pm SEM) of 11 facial samples and six nonacne-prone samples. The OX/RED ratio in nonacne-prone samples was significantly higher than the ratio obtained in homogenates from nonacne-prone areas ($p = 0.05$, paired t test, $\alpha = 0.05$).

noted using primers to the type 4 sequence (Fig 3, lane 5). No signal was noted in the control lane wherein SG RNA was run in the PCR reaction without prior reverse transcription (Fig 3, lane 6). Each of the bands noted were cut from the gel. DNA was isolated, cloned into a pCR2.1 vector (Invitrogen, Carson City, CA), and sequenced, which revealed homology of the 121 bp fragment of the type 1 reaction with the target type 1 sequence; the 151 bp fragment of the type 2 reaction with the target type 2 sequence; the 211 bp fragment with the target type 3 sequence and homology of the 181 bp fragment with the target sequence for the type 4 isozyme. The faint 120 bp band in the lane for the type 3 isozyme was sequenced and revealed only primer fragments. Remaining sequences from the type 1 reaction were not homologous with published sequences as reported in Genbank.

Oxidative activity of 17 β -HSD was greater in SG from nonacne-prone regions In addition to the seven homogenates from facial areas depicted in Table II, an additional four homogenates from acne-prone regions of skin were prepared by pooling 100–200 SG each from male cheek, male nose, male forehead, and male scalp. Six homogenates from nonacne-prone regions were prepared in the same way by pooling 100–200 SG from each of the following areas: female breast (two), female abdomen (one), male leg (one), male distal arm (two). These homogenates from acne-prone and nonacne-prone regions were assayed in duplicate for oxidative and reductive 17 β -HSD activity using C-19 steroids as substrates. The oxidative activity of 17 β -HSD (mean \pm SEM) in SG homogenates from facial areas was 18.7 ± 4.3 ($n = 11$), compared with 33.5 ± 6 ($n = 6$) in homogenates obtained from nonfacial areas (Fig 4). Although the mean oxidative activity is higher in homogenates from nonfacial areas compared with facial areas, this difference did not reach statistical significance ($p = 0.11$). There were no regional differences noted in the reductive activity of 17 β -HSD. The OX/RED ratio (mean \pm SEM) of 17 β -HSD in nonacne-prone regions, however, was significantly higher than the ratio obtained in glands from facial regions (2.53 ± 0.7 vs 1.56 ± 0.4 , $p = 0.03$, paired t test, $\alpha = 0.05$).

13-cis RA does not inhibit 17 β -HSD in SG No inhibition of 17 β -HSD oxidative activity was noted at any of the concentrations of

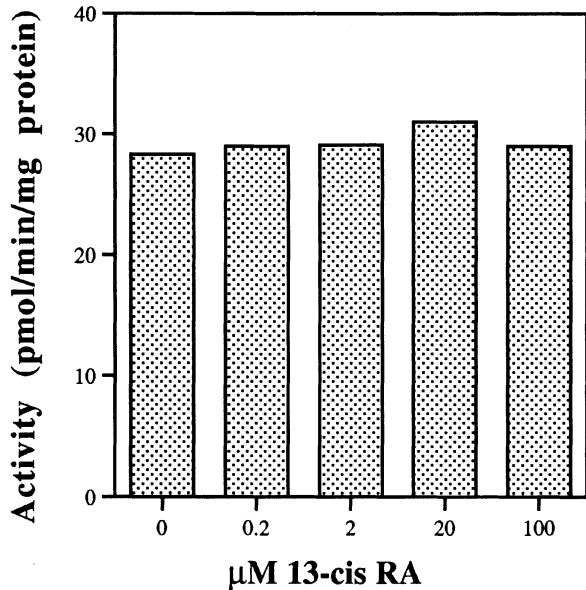


Figure 5. 13-cis RA does not inhibit 17β-HSD activity in human SG. Sebaceous glands from female breast skin were pooled, homogenized, and assayed for oxidative activity of 17β-HSD using 10 μM testosterone as a substrate in the presence of varying concentrations of 13-cis RA. Data represent the mean of two determinations.

13 cis-RA (0–100 μM) examined in a homogenate of pooled SG from female breast skin (Fig 5).

Oxidative activity of 17β-HSD is more predominant in intact SG compared with homogenized SG Activity of 17β-HSD was assayed in seven separate samples of intact SG obtained from facial regions corresponding to sites from which the homogenates were prepared for the substrate preference studies (see above). Overall, the specific activity of 17β-HSD was lower in intact glands compared with SG homogenates (Table II). As in the case of the homogenized glands, C-19 and C-18 steroids were preferred over C-21 steroids as substrates in intact glands. Oxidative activity of 17β-HSD was preferred over reductive activity in intact SG. The OX/RED ratio (mean ± SEM) was significantly greater in intact glands than in homogenized glands (paired *t* test, *p* = 0.03, α = 0.05) (Table II).

DISCUSSION

Although androgens stimulate sebum production; serum levels of androgens are normal in most acne subjects. Therefore, the local production of androgens via steroid metabolizing enzymes may be important in regulating sebum production and the development of acne. The enzyme 5α-reductase is active in SG where it converts testosterone to the more potent androgen, dihydrotestosterone, which is thought to stimulate sebum production. SG from acne-prone areas demonstrate greater activity of 5α-reductase compared with SG from nonacne-prone areas (Thiboutot *et al*, 1995). Because 17β-HSD uses testosterone as a substrate it may limit the availability of testosterone as a substrate for 5α-reductase. In this regard, oxidative activity of 17β-HSD may protect the SG from the effects of potent androgens and estrogens. A similar hypothesis has been proposed regarding the function of the type 2 isozyme of 17β-HSD in other endocrine target tissues (Andersson and Moghrabi, 1997).

Each isozyme of 17β-HSD has a unique biochemical profile. In our study, C-19, C-18, and C-21 steroids were used as substrates for both the oxidative and the reductive activity of 17β-HSD in SG from a variety of areas. The enzyme preferred to oxidize androgens and estrogens at alkaline pH using NAD as a cofactor. It also exhibited 20α-hydroxylase activity. These biochemical data are most consistent with that of the type 2 isozyme of 17β-HSD (Wu *et al* 1993). The activity of 17β-HSD was previously studied in SG using C-19 steroids as substrates only (Hay and Hodgins, 1978; Itami and Takayasu, 1981;

Hay *et al*, 1982). These studies were done prior to the identification of the six isozymes of 17β-HSD. Oxidation of testosterone was the preferred reaction at an alkaline pH optima in the presence NAD⁺ as a cofactor. This profile is also most consistent with that of the type 2 isozyme.

Northern analysis detected transcripts for the types 2 and 4 isozymes but not for the types 1 or 3. The membrane was not probed with sequences for the types 5 or 6 isozymes as the human sequences are not yet available. Biochemical data, however, indicate that it is unlikely that the type 5 isozyme is active in SG. The possibility of activity of the type 6 isozyme cannot yet be excluded.

RT-PCR amplification of target sequences in the types 1 and 2 isozymes have been reported in human sebaceous glands (Courchay *et al*, 1996). The primers chosen in our study resulted in successful amplification of sequences homologous to the types 1, 2, 3, and 4 isozymes in SG obtained from facial areas. These data indicate that transcription of the genes encoding the types 1–4 isozymes of 17β-HSD can take place in human SG. Our northern and biochemical data suggest that the type 2 isozyme predominates at both the message and the protein level. Similar observations have been made in the ovary and in meningiomas, wherein transcripts for the biochemically predominant isozyme were noted by both northern analysis and RT-PCR. Transcripts for less prominent isozymes in these tissues were detected only by RT-PCR (Carsol *et al*, 1996; Zhang *et al*, 1996).

In addition to tissue-specific differences in expression, the activity of 17β-HSD isozymes can be locally regulated by factors such as pH and substrate and cofactor availability. The oxidative activity of 17β-HSD is most tightly controlled by pH with a sharp increase in its activity as physiologic pH is approached, suggesting that oxidation predominates *in vivo*. Substrate availability can be influenced by the serum concentrations of androgen precursors and by the activity of other steroid metabolizing enzymes. The cofactor NAD is 2–3-fold more prevalent than NADP(H) in SG (Im and Hoopes, 1974). This would also favor the oxidative activity of 17β-HSD and thereby limit availability of the more potent C-19 and C-18 steroids.

In SG from acne-prone areas, the OX/RED ratio of 17β-HSD is significantly less than the ratio in SG from nonacne-prone areas, suggesting that more testosterone is being made available for conversion to dihydrotestosterone by 5α-reductase in acne-prone areas. These data, in combination with data demonstrating increased 5α-reductase activity in facial SG, support the hypothesis that regional differences exist in the capacity of SG to metabolize androgens (Thiboutot *et al*, 1995). SG from acne-prone areas may be programmed to produce larger quantities of potent androgens and hence more sebum. It is possible that these differences may be magnified in skin affected by acne.

Several investigators have stressed the importance of studying the activity of 17β-HSD in intact cells rather than in homogenates. Enzyme activity in intact cells depends upon the availability of endogenous cofactors, whereas cellular homogenates are supplied with excess concentrations of cofactor. The cellular compartmentalization and intracellular pH gradient are disrupted in homogenates as well. Our data demonstrate that the oxidative activity of 17β-HSD is preferred in both cellular homogenates and intact SG. The oxidative to reductive ratio of 17β-HSD was several fold higher in intact glands than in SG homogenates for both C-19 and C-18 steroids, indicating that the dominant oxidative activity of 17β-HSD is even more pronounced in intact cells. This is in concordance with the observation that the magnitude of the differences in the oxidative and reductive activities of 17β-HSD are amplified in intact cells compared with homogenized cells. From a technical standpoint, however, assay of intact glands is more difficult as glands need to be dissected from fresh tissue and directly assayed.

The activity of 17β-HSD can be regulated by retinoids, growth factors, and cytokines (Duncan *et al*, 1994; Ghersevich *et al*, 1994; Reed *et al*, 1994). 13-cis-RA has been reported to competitively inhibit activity of 17β-HSD in lysates of human embryonic kidney cells transfected with the type 6 isozyme (Biswas and Russell, 1997). No direct inhibition of type 2 17β-HSD activity by 13-cis-RA was noted in our study. These data lend further support to the hypothesis that a reduced serum level of androgens in subjects treated with 13-

cis RA results from a reduction in the amount of SG tissue capable of producing androgens rather than by inhibition of steroid metabolizing enzymes (Lookingbill *et al*, 1992).

Although androgens have been the focus in the hormonal regulation of the SG, systemic administration of estrogens is known to inhibit sebum production (Pochi and Strauss, 1976). It is not known if estrogens oppose the effects of androgen on sebum production directly or via a feedback mechanism. The demonstration that the type 2 isozyme of 17 β -HSD can interconvert weak and potent C-19 and C-18 steroids within the SG supports the concept that this enzyme may regulate the net effect of sex steroids locally.

In conclusion, 17 β -HSD may be a gatekeeping enzyme that regulates the balance of potent androgens and estrogens within the SG. In normal SG, the type 2 isozyme predominates resulting in backconversion of C-19 and C-18 steroids to less potent forms. Because the net effect of 17 β -HSD activity can be modulated by serum levels of precursor steroids, cytokines, retinoids, and the redox state of the tissue, its directional activity may differ in skin affected by acne. Agents that further promote the oxidative activity of 17 β -HSD or inhibit its reductive function may be of value in the treatment of hormonally regulated skin diseases such as acne.

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