Characterization of a Partial cDNA for Lysyl Hydroxylase from Human Skin Fibroblasts; Lysyl Hydroxylase mRNAs Are Regulated Differently by Minoxidil Derivatives and Hydralazine

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Lysyl hydroxylase (LH) is an essential enzyme in collagen biosynthesis that catalyzes the formation of hydroxylysine required for intermolecular crosslinking of collagen. We have isolated a partial (2.2-kb) cDNA for LH from human skin fibroblasts using PCR. DNA sequencing revealed 72% homology of the human coding sequence with the chick LH sequence at the nucleotide level and 76% homology predicted at the amino acid level. The LH cDNA hybridized strongly with two mRNA species of 2.4 and 3.4 kb on Northern blots of normal fibroblast RNA. Administration of minoxidil decreased both mRNA species without affecting levels of the mRNAs for the β subunit of prolyl 4-hydroxylase (PH) or α1(I) collagen. Two derivatives of minoxidil (3′ hydroxyminoxidil and 4′ hydroxyminoxidil) produced similar decreases in LH mRNAs. In contrast hydralazine increased the mRNAs for LH in parallel with its previously reported effect on the mRNA for the β subunit of PH. This effect is accompanied by virtual elimination of the α1(I) collagen mRNAs. These results on the action of minoxidil and hydralazine at the pretranslational level correlate well with their previously reported effect on enzyme activity and collagen biosynthesis and indicate that changes in steady-state mRNA levels can account directly for changes at the protein level. Moreover, the unique action of minoxidil in specifically decreasing LH mRNAs contrasts with the less specific stimulatory effects of hydralazine and suggests that these pharmaceuticals are regulating expression of LH at a pretranslational level by different mechanisms. *J Invest Dermatol 99:864–869, 1992*

Lysyl hydroxylase (LH), which catalyzes the hydroxylation of lysine residues in peptide linkages, is a key post-translational modifying enzyme in collagen biosynthesis [1]. Hydroxylysine residues play a central role in collagen stability as they are essential for the formation of covalent intermolecular crosslinks and also as specific sites for glycosylation [2]. The importance of LH is demonstrated in patients with the type VI variant of Ehlers-Danlos syndrome (EDS VI), characterized biochemically by a deficiency in LH activity [3,4] and clinically by muscle hypotonia, joint laxity, moderate to severe kyphoscoliosis, and skin that is soft, distensible, and easily bruisable [1,5].

The present study describes the isolation and sequencing of a partial cDNA for LH. Based on the recently published sequence of the cDNA for chick LH [6] we successfully amplified a 2.2-kb cDNA for LH from human skin fibroblasts using PCR. This has enabled us to study the pharmaceutically mediated regulation of LH at the molecular level. LH requires the co-factors O_2, ferrous iron, α ketoglutarate, and ascorbate for its catalytic activity [2,4]. Similar co-factors are required by prolyl 4-hydroxylase (PH) [1], another important post-translational modifying enzyme in collagen biosynthesis. Previous studies in our laboratory have shown that administration of minoxidil, an antihypertensive drug that also stimulates hair growth [8], to human skin fibroblasts has the unique effect of decreasing LH activity [4]. It does not affect the activity of PH [4], or significantly decrease collagen biosynthesis.* The specificity of this effect suggests that minoxidil may directly suppress the synthesis of LH in the cell. Two metabolites of minoxidil (3′-hydroxyminoxidil and 4′-hydroxyminoxidil) that do not possess such severe antihypertensive activity as minoxidil, and are more water soluble, are as effective as the parent compound in specifically decreasing the activity of LH in cultured dermal fibroblasts [9]. In contrast, the antihypertensive drug hydralazine increases both LH and PH activity in human skin fibroblasts and causes a dramatic decrease in collagen biosynthesis [10]. Although this significant decrease suggests a potential use as an antifibrotic agent, the concentration of hydralazine in the plasma of hypertensive patients treated with this drug

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Abbreviations:
- DMEM: Dulbecco's modified Eagle's medium
- DTT: dithiothreitol
- EDS VI: Ehlers-Danlos syndrome type VI
- kb: kilobase pair
- LH: lysyl hydroxylase
- nt: nucleotide
- PCR: polymerase chain reaction
- PH: prolyl 4-hydroxylase
- SAH: S-adenosylhomocysteine hydrolase
- SDS: sodium dodecyl sulfate
- SSC: saline-sodium citrate buffer

* S. Murad, personal communication.

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[11] is tenfold lower than that administered to fibroblasts, which makes it unlikely that hydralazine can be used orally to prevent fibrosis.

In this study we have used the cDNA for human LH to study the pharmaceutically mediated regulation of this important enzyme at the mRNA level in dermal fibroblasts. We have shown that the unique effect of minoxidil in decreasing the activity of LH is a direct result of changes in steady-state mRNA levels. Moreover, this effect appears unrelated to levels of the mRNA for the β subunit of PH or α1(I) collagen mRNAs. In contrast the hydralazine-mediated increase in LH mRNAs is accompanied by an increase in β PH mRNA and a concomitant decrease in α1(I) collagen mRNAs, suggesting that these pharmaceuticals are regulating expression of LH mRNAs by different mechanisms.

MATERIALS AND METHODS

Cell Culture Normal skin fibroblasts were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% heat-inactivated calf serum as previously described [12]. In the time-course experiments, cells were transferred to DMEM supplemented with 0.5% dialyzed fetal bovine serum in the presence of 100 μM ascorbate and treated with 50 μM hydralazine, 500 μM minoxidil, or its 3′ or 4′ hydroxy derivatives [9] and harvested at 0, 3, 24, 48, 72, and 96 h. The media were changed for all plates at each treatment point.

RNA Isolation Total RNA was isolated from approximately 6×10^6 cells per treatment point by the acid-guanidinium thiocyanate-phenol-chloroform extraction method [13]. Poly(A)RNA was prepared using oligo(dT)-cellulose columns (type 3, Collaborative Research) [14]. Total RNA (5 μg) or poly(A)RNA (1 μg) was separated electrophoretically on denaturing 1% agarose gels [15], transferred overnight to GeneScreen Plus membrane (New England Nuclear) in 10 × SSC transfer buffer and baked for 2 h at 80°C.

Blot Analysis The membrane blots were hybridized with cDNA probes for a) the human proα1 chain of type I collagen [16], which detects both 4.8- and 5.8-kb mRNAs of α1(I) collagen; b) the β subunit of prolyl 4-hydroxylase [17], which hybridizes with a single mRNA species of 2.5 kb; c) lysyl hydroxylase (this study); and d) S-adenosylhomocysteine hydrolase (SAH) [18], which hybridizes with a 2.6-kb mRNA. The cDNA probes were labeled with [α-^{32}P]dCTP by the random primer method to a specific activity of 5×10^8 cpm/μg [19]. The blots were prehybridized at 42°C in 50% formamide, 1 M NaCl, 10% dextran sulfate, 0.5% SDS, and 10 μg/ml sonicated salmon sperm DNA and hybridized with the radiolabeled probe overnight under the same conditions. Blots were washed twice using 2 × SSC for 5 min at room temperature, then washed in 2 × SSC, 1% SDS twice for 30 min at 42°C. Following autoradiography, the mRNAs were quantitated by scanning with an LKB ultrascan laser densitometer and normalized with respect to SAH mRNA. Each experiment was repeated at least twice. Results are expressed as percentage of control for each mRNA.

Primers The first-strand cDNA synthesis primer was the oligo dT_{17}-adapter primer described by Frohman *et al*, a 35mer with the sequence 5′GACTCGAGTCGACATCGAT$_{17}$ 3′ [20]. The 3′ PCR primer was the 18mer containing the adapter primer sequence 5′ to dT_{17}. The 5′ PCR primer was a 20mer, based on the sequence of chick LH cDNA, nt 862-881, 5′ACCAAGCTGCAGCTGAACTA 3′ [6].

First-Strand cDNA Synthesis and Polymerase Chain Reaction Poly(A) RNA was isolated from human skin fibroblasts and first-strand cDNA was performed as described [21] with 5 μg poly(A)RNA and 625 ng of oligo dT_{17} adapter primer [20]. Amplification of the 2.2-kb cDNA for LH was performed in a 100-μl reaction containing 20 ng cDNA, 60 ng of the 5′ and 3′ amplification primers described above, 200 μM of each deoxynucleotide, 1.5 mM Mg^{++}, 50 mM KCl, 10 mM DTT, 10 mM Tris-HCl, pH 8.3, 0.01% gelatin, and 2.5 units Taq polymerase (Perkin-Elmer Cetus). The reactions were run for 40 cycles with denaturation at 94°C for 1 min 10 seconds, annealing at 50°C for 2 min 10 seconds, and extension at 72°C for 6 min in a DNA Thermal Cycler (Perkin-Elmer Cetus). After each cycle, 10 additional seconds were added to the extension step. On completion of the PCR run, 20 μl of each reaction mixture were electrophoresed on a 1% agarose-ethidium bromide gel, visualized under UV light, and photographed. These gels were then hybridized *in situ*.

In Situ Gel Hybridization The gels were denatured at 4°C for 30 min in 0.5 N NaOH, 1.5 M NaCl, then neutralized at 4°C for 30 min in 1 M Tris, pH 7.5, 1.5 M NaCl and dried under vacuum at 60°C. The gels were rehydrated in deionized H_2O and prehybridized for 2 h at 68°C [22] in 150 mM NaCl, 15 mM Tris-HCl, pH 7.5, and 1 mM EDTA. Hybridization was performed for 2 h at 68°C in the same buffer with the addition of 0.1% SDS and the 2.2-kb cDNA for LH excised from another gel and labeled with [α-^{32}P]dCTP by the random primer method to a specific activity of 5×10^8 cpm/μg [19]. The gels were washed with 2 × SSC at 68°C for 2 h, and with 1 × SSC at 68°C for 1 h, followed by autoradiography.

Amplification and Cloning of the 2.2-kb LH cDNA The 2.2-kb cDNA amplified by PCR was excised from a low-melting-point agarose gel and purified by the Glassmax DNA spin cartridge system (Gibco-BRL). The DNA was restricted with Pvu II and Sal I (restriction sites present in the 5′ and 3′ amplification primers, respectively) to give a 1.8-kb fragment. This shortened DNA was the result of an additional Pvu II restriction site at position 1247 (numbering based on the chick cDNA sequence), approximately 400 bases downstream from the 5′ amplification primer sequence.

The 1.8-kb fragment was purified by gel electrophoresis and Glassmax (Gibco-BRL) and ligated into plasmid pSP72 (Promega) that had been restricted with Pvu II and Sal I and dephosphorylated with bovine alkaline phosphatase (Gibco-BRL). The constructs were transformed into *Escherichia coli* strain DH5α (Gibco-BRL) and positive clones identified by hybridization with the 2.2-kb LH cDNA. The isolated DNA was purified by CsCl gradient centrifugation [15] prior to sequencing.

Sequencing Methods The 1.8-kb cDNA was sequenced by the Sanger dideoxy method [23] using the Sequenase kit (U.S. Biochemicals) according to the manufacturer's recommendations.

The remaining 400 bases in the 2.2-kb cDNA were sequenced by the ds DNA cycle sequencing system (Gibco-BRL). Approximately 5–10 ng of the gel-purified 2.2-kb cDNA were used in each sequencing reaction with 14 ng of primer (labeled at the 5′ end with [γ-^{32}P]ATP according to the manufacturer's instructions). The PCR reactions were run for 30 cycles with denaturation at 95°C for 1 min, annealing at 50°C for 2 min, and extension at 70°C for 2 min in a DNA Thermal Cycler.

RESULTS

Amplification of a 2.2-kb cDNA for LH by PCR Prior to amplification, we synthesized first-strand cDNA from poly(A)-RNA isolated from human skin fibroblasts using the oligo dT_{17} adapter primer described by Frohman *et al* [20]. Several 20 nucleotide primers based on the recently published sequence of chick LH cDNA [6] were synthesized to serve as potential 5′ primers to amplify a cDNA for human LH. One primer based on nt 862–881 of the chick sequence for LH was successfully used as 5′ primer to amplify LH cDNA from first-strand cDNA in a PCR reaction using the adapter sequence of the oligo dT adapter primer [20] as the 3′ primer. This primer contains several possible cloning sites as shown in Fig 1. Two restriction sites, Pvu II and Pst I, are present in the 5′ amplification primer.

Electrophoresis of the PCR reaction showed three bands (Fig 2*A*), a major band at 2.2 kb, and minor bands at 1.6 and 1.7 kb. Each of these bands hybridized *in situ* with the 2.2-kb cDNA excised from another gel and labeled, showing their relatedness to each other (Fig 2*B*). These transcripts may represent co-linear cDNAs with 3′ untranslated regions of different lengths. Utilization of

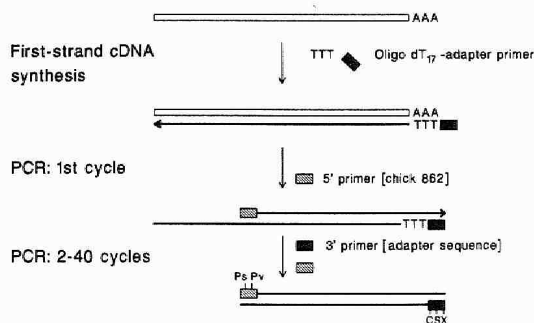


Figure 1. Strategy for synthesis and amplification of a 2.2-kb partial cDNA for human lysyl hydroxylase using PCR. The 5′ amplification primer (chick 862) was based on sequence nt 862–881 of chick LH cDNA [6] and the 3′ amplification primer was the adapter primer sequence 5′ to the oligo dT_{17} [20] used to prime first-strand cDNA synthesis. The amplification product contains at its 5′ end a Pst 1 (Ps) and a Pvu II (Pv) restriction site and at its 3′ end the Cla I (C), Sal I (S), and Xho I (X) restriction sites to aid cloning.

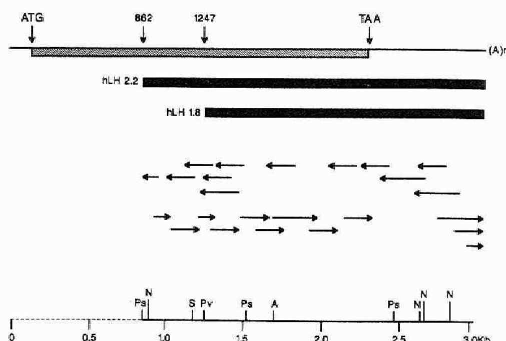


Figure 3. Restriction endonuclease map and sequencing strategy for cDNAs representing human lysyl hydroxylase from skin fibroblasts. *Arrows,* directions and lengths of the sequencing runs. A composite map of the coding *(hatched)* and noncoding sequences is given at the *top.* The codons ATG and TAA represent the start and stop signals for translation. nt 862 represents the location of the 5′ primer used to amplify the 2.2-kb cDNA and nt 1247 represents the Pvu II restriction site used in cloning the 1.8-kb cDNA. A partial restriction map is given (Ps, Pst I; N, Nco I; S, Sau I; Pv, Pvu II; A, Aha II). *Dotted line,* 5′ end of the cDNA that has not yet been sequenced.

different polyadenylation sites has been reported for both the proα1(I) collagen gene, which transcribes two distinct mRNAs of approximately 4.8 and 5.8 kb [16], and the proα2(I) collagen gene, which transcribes three to five mRNAs of between 4.1 and 5.7 kb [24]. We confirmed that we had isolated the correct cDNA for LH by comparing a short cycle-sequenced (Gibco-BRL) fragment of the 2.2-kb cDNA with the corresponding chick LH cDNA sequence (data not shown).

Sequence Strategy and Analysis The sequencing strategy for the 2.2-kb cDNA for LH is outlined in Fig 3. Restriction of the 2.2-kb cDNA with Pvu II/Sal I gave a fragment of approximately 1.8 kb due to the presence of an additional Pvu II restriction site, not present in the chick LH cDNA [6], 385 bases downstream from the 5′ end of the 2.2-kb cDNA at the human equivalent of nt 1247 of chick LH cDNA. The 1.8-kb cDNA was ligated into plasmid pSP72 (Promega) restricted with the same enzymes and amplified as described in *Materials and Methods.* Following CsCl gradient centrifugation [15], the 1.8-kb cDNA was sequenced by the Sanger dideoxy method [23]. The remaining 385 bases at the 5′ end of the 2.2-kb cDNA were cycle sequenced (Gibco-BRL).

Sequence analysis of the partial 2.2-kb cDNA for LH showed homology of 72% with chick LH at the DNA level and a predicted homology of 76% at the amino acid level (data not shown). The cDNA had a noncoding region of 728 nucleotides, excluding the poly(A) tail. A slightly atypical polyadenylation signal ATTAAA was present 13 nt upstream from the poly(A) tail. Nine cysteine residues predicted from the partial human cDNA sequence were conserved between chick and human LH. In addition, although the chick sequence nt 862–881 containing a Pvu II site was successfully used to amplify the human cDNA for LH, a single base difference in the 20mer sequence (a T in the human sequence replacing a C in the chick sequence at nt 874) precluded the presence of a Pvu II site in this region of the human cDNA.

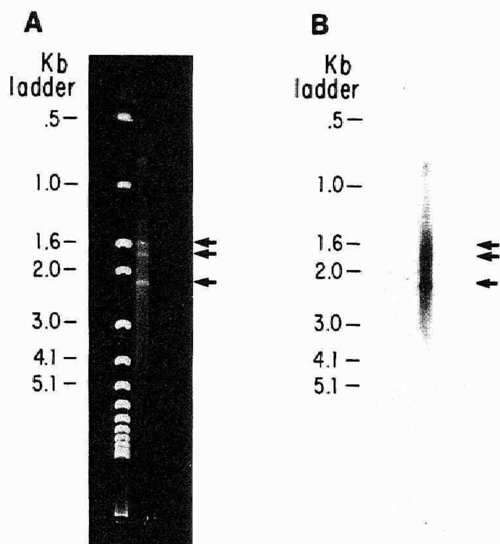


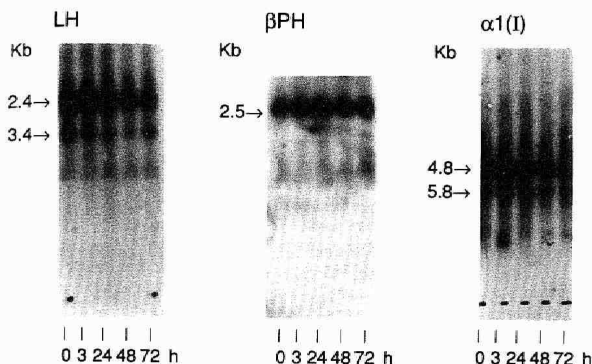
Figure 2. Structural relatedness of three major products from PCR amplification of human skin fibroblast cDNA. *A,* three PCR products of 2.2, 1.7, and 1.6 kb (shown by *arrows*) amplified from human skin fibroblast cDNA were separated by ethidium bromide–agarose gel electrophoresis. *B, in situ* hybridization of the same gel with the major 2.2-kb product excised from another gel and ^{32}P-labeled (see *Materials and Methods*).

Northern Blot Analysis of Regulation of LH The labeled 2.2-kb cDNA for LH, which includes approximately 1.5 kb of coding sequence and the entire 3′ noncoding sequence, hybridized strongly with two mRNAs of 2.4 and 3.4 kb in untreated dermal fibroblasts (Fig 4). Minoxidil decreased levels of both mRNAs for LH in a 72-h time course; the 3.4-kb mRNA was reduced to 30% of control by 24 h, whereas the 2.4-kb mRNA was decreased more slowly to 50% of control by 72 h. Minoxidil administration did not affect the mRNAs for the β subunit of PH or α1(I) collagen, demonstrating its specificity for LH at the mRNA level (Fig 4*A*). In contrast, hydralazine increased the mRNAs for LH two to threefold and the mRNA for βPH threefold (Fig 4*B*), whereas, as previously shown [25], it virtually eliminated the 4.8- and 5.8-kb mRNAs for α1(I) collagen within 72 h.

These results correlate with earlier studies in skin fibroblasts [4], demonstrating that minoxidil specifically decreases LH activity without significantly affecting PH activity or collagen biosynthesis. Furthermore, the previously reported effect of hydralazine in stimulating both LH and PH activity while virtually eliminating collagen biosynthesis in dermal fibroblasts [10] appears to be a direct result of hydralazine-mediated changes in their respective steady-state mRNAs.

In addition, both 3′-hydroxyminoxidil and 4′-hydroxyminoxidil

A. Minoxidil



B. Hydralazine

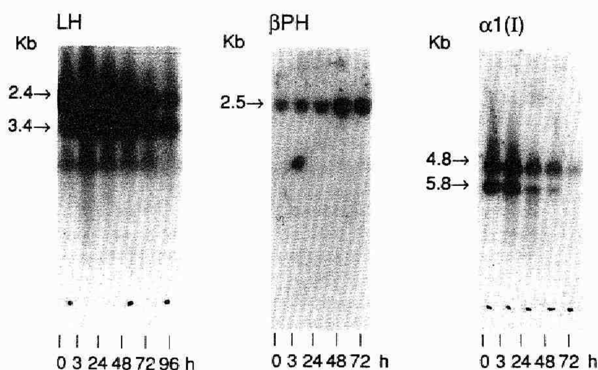


Figure 4. Northern blot hybridizations showing effect of *(A)* minoxidil and *(B)* hydralazine on the mRNAs for LH, the β subunit of PH, and $\alpha 1$(I) collagen between 0 and 72 h. Total RNA was isolated from fibroblasts cultured with minoxidil (500 μM) or hydralazine (50 μM) at the times shown. As described in *Materials and Methods*, 5-μg samples were electrophoresed, then transferred to GeneScreen Plus membranes, and blots were individually hybridized with a) the 2.2-kb cDNA probe for LH (which detects both 2.4- and 3.4-kb mRNAs); b) a cDNA probe for the β subunit of PH (which detects a 2.5-kb mRNA), and c) a cDNA probe for $\alpha 1$(I) collagen (which detects both 4.8- and 5.8-kb mRNAs).

produced decreases in LH mRNAs similar to that produced by minoxidil, an observation that contrasted with the stimulatory effect of hydralazine as shown in Table I. As observed with minoxidil, these minoxidil derivatives produce a corresponding decrease in LH activity [9].

DISCUSSION

This study describes the isolation of a 2.2-kb partial cDNA for LH from human skin fibroblasts using PCR, which has enabled us to study the mechanisms involved in the regulation of LH by the pharmaceuticals minoxidil and hydralazine.

Sequence analysis of this partial human cDNA indicates a homology of 72% with chick LH at the DNA level and a predicted homology of 76% at the amino acid level. This is somewhat lower than the homology reported between the chick and human sequences for the α subunit of prolyl hydroxylase [26,27], 81% and 88% at the DNA and amino acid level, respectively. Our human cDNA for LH has a noncoding region of 728 nucleotides that is 853 nucleotides shorter than the corresponding noncoding sequence of the chick cDNA [excluding the poly(A) tails] [6].

During the preparation of this manuscript Hautala *et al* [28] published the cloning of lysyl hydroxylase isolated from human placenta. To date, a comparison of our fibroblast cDNA sequence with that of the human placental cDNA reveals six single base differences.* Whether these changes are the result of simple polymorphisms or whether they can be attributed to tissue specificity remains to be determined. It appears likely that a family of lysyl hydroxylases may exist, each with its own tissue specificity and collagen-type specificity. For example, in certain patients with EDS VI, it has been shown that collagens from skin (primarily types I and III) are hydroxylysine deficient but collagen from cartilage (primarily type II) is not [1,29]. In addition, in one individual with EDS VI, collagen from skin and tendon was hydroxylysine-deficient, whereas collagen in bone was not [29]. As the predominant form of collagen in skin, tendon and bone is type I, this would indicate the existence of tissue-specific lysyl hydroxylases. Further analysis is necessary to determine whether our cDNA isolated from skin represents a species of lysyl hydroxylase that is distinct from the LH cDNA isolated from placenta.

The 2.2-kb cDNA for LH hybridizes strongly with two mRNAs of approximately 2.4 and 3.4 kb on Northern blots of total or poly(A) RNA isolated from normal human skin fibroblasts. Both these mRNAs appear to be regulated by the pharmaceuticals minoxidil and hydralazine. Previous studies in our laboratory [4] have shown that the antihypertensive drug minoxidil dramatically reduces the level of LH activity in dermal fibroblasts. The specificity of this decrease is demonstrated by the lack of effect of minoxidil on prolyl hydroxylase activity [4] and a minimal reduction of collagen biosynthesis. In the present study we demonstrate that this unique effect of minoxidil is a direct result of decreases in steady-state levels of LH mRNAs. The lack of any significant effect of minoxidil on PH activity and collagen biosynthesis is reflected in the unchanged steady-state mRNA levels for the β subunit of PH and $\alpha 1$(I) collagen.

The specificity of this effect on LH by minoxidil is also demonstrated by two metabolites of minoxidil (3′-hydroxyminoxidil and 4′-hydroxyminoxidil). The present study demonstrates that the suppression of LH by these hydroxy derivatives of minoxidil results

Table I. Effects of Minoxidil, 3′- and 4′-Hydroxyminoxidil and Hydralazine on the 2.4- and 3.4-kb mRNAs for LH Between 0 and 72 h[a]

Treatment	LH mRNAS (Kb)	RNA (percent of control) 0 h (control)	3 h	24 h	48 h	72 h
Minoxidil						
—	2.4	100	88	71	54	50
—	3.4	100	45	27	23	28
3′-OH minoxidil						
—	2.4	100	113	78	57	78
—	3.4	100	142	76	39	48
4′-OH minoxidil						
—	2.4	100	107	97	66	61
—	3.4	100	62	48	23	34
Hydralazine						
—	2.4	100	189	139	69	86
—	3.4	100	269	239	168	146

[a] Total RNA was isolated from fibroblasts treated with minoxidil (500 μM), 3′- and 4′-hydroxyminoxidil (each at 500 μM), and hydralazine (50 μM) at the times shown and levels of mRNAs quantitated by hybridization with the 2.2-kb LH cDNA as described in *Materials and Methods*. Values were normalized with respect to SAH mRNA. The results are expressed as percentages of control for each message and represent the mean of at least two experiments in which each mRNA was analyzed in duplicate.

* Yeowell HN, Ha V, Pinnell SR (manuscript submitted). GenBank accession number: M98252.

directly from decreased steady-state mRNA levels of LH in a manner similar to that of the parent compound.

Recently, Hautala *et al* [30] have reported a similar decrease in an mRNA for LH using their cDNA for human placenta [28] in Northern blot hybridization of RNA from minoxidil-treated human skin fibroblasts. In contrast to our observations, however, their cDNA probe only detects a single mRNA of 3.2 kb in dermal fibroblasts. This may be the result of differences in the cDNA probes used for hybridization studies. Our 2.2-kb cDNA, which includes both 1.5 kb of coding region and the entire 728 nucleotide 3′ noncoding sequence, detects two mRNAs of 2.4 and 3.4 kb, whereas preliminary studies in our laboratory (unpublished observations) indicate that a 1.5-kb cDNA that includes only coding region hybridizes to one mRNA species of 3.4 kb. This suggests that the 3′ noncoding region of LH may be hybridizing to a 2.4-kb mRNA unrecognized by the coding region. Additional studies to explain this intriguing observation are presently underway in our laboratory.

Administration of hydralazine, another pharmaceutical used to treat hypertension, increased levels of steady-state mRNAs for LH in skin fibroblasts, an observation consistent with its previously reported stimulation of LH activity [10]. However, unlike the specificity of minoxidil for LH, hydralazine also affects the mRNA for the β subunit of PH, increasing it in parallel with its effect on enzyme activity. In addition, hydralazine causes a significant decrease in $\alpha 1$(I) collagen mRNAS [25], virtually eliminating both mRNAs within 72 h. The low level of collagen expressed in hydralazine-treated fibroblasts has been shown to be severely deficient in hydroxyproline and hydroxylysine [10]. Thus the overproduction of the hydroxylating enzymes LH and PH in these cells may represent a compensatory mechanism turned on by the decreased synthesis of procollagen, which is severely deficient in hydroxyproline and hydroxylysine. In contrast, minoxidil only slightly decreases the rate of collagen synthesis, but the collagen produced is hydroxylysine deficient [31]. Minoxidil appears to act by directly suppressing LH at the pretranslational level, thereby decreasing its activity required for hydroxylation of specific lysyl residues in collagen, whereas hydralazine may regulate LH and PH activities via an indirect mechanism coupled to synthesis of $\alpha 1$(I) collagen mRNAs.

In summary, we have isolated a cDNA for lysyl hydroxylase from human skin fibroblasts, which has enabled us to study the mechanism of regulation of lysyl hydroxylase by such pharmaceuticals as minoxidil, its hydroxy derivatives, and hydralazine. We have demonstrated that the unique suppression of LH activity in cultured fibroblasts by minoxidil and derivatives is a direct result of changes in the steady-state levels of mRNAs for this enzyme, an effect that appears unrelated to levels of mRNAs for βPH or $\alpha 1$(I) collagen. In contrast, the increase in LH mRNAs by hydralazine treatment is accompanied by an increase in βPH mRNA and a dramatic decrease in $\alpha 1$(I) collagen mRNAs. It therefore appears that these pharmaceuticals are regulating expression of LH at a pre-translational level by different and seemingly independent mechanisms.

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