# Activation of Cytoprotective Prostaglandin Synthase-1 by Minoxidil as a Possible Explanation for Its Hair Growth–Stimulating Effect

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**Data from the literature indicate that nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, naproxen, piroxicam, or ibuprofen, induce hair loss *in vivo*. These NSAIDs are well-known inhibitors of both the cytoprotective isoform of prostaglandin endoperoxide synthase-1 (PGHS-1) and of the inducible form (PGHS-2). By immunohistochemical staining, we found that PGHS-1 is the main isoform present in the dermal papilla from normal human hair follicle (either anagen or catagen), whereas PGHS-2 was only faintly and exclusively expressed in anagen dermal papilla. Thus, PGHS-1 might be the primary target of the hair growth–inhibitory effects of NSAIDs. We thus speculated that activation of PGHS-1 might be a mechanism by which minoxidil (2,4-diamino-6-piperidinopyrimidine-3-oxyde) stimulates hair growth *in vivo*. We demonstrate here that minoxidil is a potent activator of purified PGHS-1 (AC50 = 80 $\mu$M), as assayed by oxygen consumption and $PGE_2$ production. This activation was also evidenced by increased $PGE_2$ production by BALB/c 3T3 fibroblasts and by human dermal papilla fibroblasts in culture. Our findings suggest that minoxidil and its derivatives may have a cytoprotective activity *in vivo* and that more potent second-generation hair growth-promoting drugs might be designed, based on this mechanism.** ***Key words: minoxidil/prostaglandin synthase/ hair growth/ cytoprotection. J Invest Dermatol 108:205–209, 1997***

Minoxidil is a pyrimidine derivative (2,4-diamino-6-piperidinopyrimidine-3-oxyde) initially developed as a potent antihypertensive agent (Dargie *et al*, 1977, Devine *et al*, 1977). In addition to being a direct-acting vasodilator, it was unexpectedly found to stimulate hair growth *in vivo* (Weiss *et al*, 1984). This major side effect in hypertensive treatment led to its clinical use for the treatment of common baldness (androgenetic alopecia) and alopecia areata. Minoxidil was later reported to stimulate a time-dependent increase in $^{3}$[H]-thymidine and $^{35}$[S]-cysteine incorporation in mouse vibrissa follicles in organ culture *in vitro* (Buhl *et al*, 1989). Although it has been recently suggested that both anti-hypertensive and hair-regrowth effects were due to the action of its sulfated metabolite (minoxidilS) on $K^+$ channels (Meisheri *et al*, 1991; Buhl *et al*, 1992a), no unifying concept explains by which biochemical mechanism(s) minoxidil can exhibit its pleiotropic effects. For example, minoxidil has also been reported to inhibit lysyl hydroxylase *in vitro* in human dermal fibroblasts (Murad *et al*, 1992) as well as in fibroblasts from the external connective tissue sheath of human hair follicles (Mahé *et al*, 1996). The exact way by which it stimulates hair growth *in vivo* is still a matter of debate.

Other drugs are known to interfere with the hair cycle. Some (e.g., antineoplastic agents, such as doxorubicin) induce an abrupt cessation of mitotic activity. Others such as $\beta$-blockers, interferons, or retinoids (Pillans and Wood, 1995) precipitate a premature resting phase (telogen effluvium). Similarly, anti-inflammatory agents such as indomethacin, naproxen, piroxicam, or ibuprofen provoke hair loss (Tosti *et al*, 1994; Pillans and Wood, 1995; for reviews). Because these compounds inhibit the prostaglandin endoperoxide synthases (PGHS-1, E.C. 1.14.99.1, and PGHS-2, E.C.1.14.99.2), which catalyze the first two steps of the arachidonic acid cascade (Smith and Marnett, 1991), it is possible that at least one of these isoforms is active in hair growth. Minoxidil has also been reported to affect the metabolism of arachidonic acid by inhibiting prostacyclin synthase in bovine aorta endothelial cells (Kvedar *et al*, 1988) or increasing prostaglandin $E_2$ ($PGE_2$) production by dermal papilla fibroblasts in culture *in vitro* (Lachgar *et al*, 1996). We thus investigated whether PGHS-1 and PGHS-2 isoforms were expressed in human hair follicles and whether minoxidil was a modulator of these two key enzymes of the arachidonic acid cascade that is involved in the generation of prostaglandins.

## MATERIALS AND METHODS

**Chemicals** $PGE_2$ was obtained from Cayman Chemical Co. (Ann Arbor, MI). Arachidonic acid and indomethacin were obtained from Sigma Chemical Company (Saint-Quentin Fallavier, France). Potassium arachidonate for assays was prepared according to the Cayman Chemicals Company procedure as follows: 100 $\mu$l of cold (0–5°C) 0.1 M potassium hydroxide were added to 95 $\mu$l of ethanol containing 4.5 mg of arachidonic acid and thoroughly mixed. Then, 2 ml $\mu$l of water (free of redox active transition metal ions) were added and mixed to obtain a clear solution of 6.7 mM potassium arachidonate that could be added directly to the assay mixture

Manuscript received May 9, 1996; revised October 18, 1996; accepted for publication October 30, 1996.

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Abbreviations: PGHS, prostaglandin-endoperoxide synthase; DP, dermal papilla.

0022-202X/97/$10.50 

(stored at 4°C protected from light). L-Glutamine, penicillin/streptomycin, and 199 medium were supplied by GIBCO (Cergy Pontoise, France).

**Biologic Materials** Ovine prostaglandin endoperoxide synthase-1 (PGHS-1) (E.C. 1.14.99.1) isolated from sheep seminal vesicles, was purchased from Cayman Chemical Co. Human scalp biopsies were obtained from female face-lifting surgery with patients' consent. They were cut into small pieces under a microscope, each piece containing about six intact follicles. Tissue samples were then coated in Tissue-Tek O.C.T. from Miles Inc. (Elkhart, IN), quick frozen over dry ice (−80°C), and stored at −80°C.

**Immunohistochemistry** Longitudinal sections (5 μm) of hair follicles were obtained with a cryostat HM 500 M (Microm, Francheville, France) set at −40°C. Sections were air dried and stored at +4°C overnight before processing. Sections were then fixed in acetone at −20°C for 10 min followed by several washes in phosphate-buffered saline. Endogenous peroxidase activity was quenched by immersing the slides in 0.1% hydrogen peroxide for 10 min. To block nonspecific binding sites and minimize background, the sections were then covered with 1% nonfat milk-phosphate-buffered saline for 15 min. The following primary antibody dilutions in phosphate buffered saline containing 10% of goat normal serum were applied to the sections for 18 h at +4°C: the mouse $IgG_{2b}$ from the cyo-1 hybridoma cell line anti-PGHS-1 monoclonal antibody (MoAb) PG21 (Oxford Biomedical Research, Oxford, MI), which was developed against ovine PGHS-1 and reacts with human PGHS-1 (DeWitt *et al*, 1982), was diluted to 1:8. The mouse $IgG_1$ (clone 33) anti-PGHS-2 MoAb C22420 (Transduction Laboratories, Lexington, KY) was diluted to 1:25. Biotinylated goat anti-mouse immunoglobulins (Dako, Trappes, France) were applied for 30 min at room temperature. Strepavidin biotin peroxidase complex was prepared as indicated by the manufacturer; sections were covered with the strepavidin biotin peroxidase complex solution for 30 min at room temperature. Substrate solution (AEC, Sigma, St. Quentin Fallavier, France) was prepared and applied for 6 min as indicated, and the color reaction was then stopped by washing with double distilled water. The slides were finally mounted with cover glasses using glycerol gelatin (Sigma) before observation under a light microscope. Because we found no staining of any compartment of the catagen hair follicle (see *Results*) with the mouse IgG1 (clone 33) anti-PGHS-2 MoAb C22420 as the primary antibody, this antibody served as a negative control showing that the biotinylated goat anti-mouse immunoglobulins were not responsible for the staining we observed using anti-PGHS-1 MoAb. Additional negative controls were performed by replacing the primary antibody with phosphate-buffered saline; they gave the expected negative results (data not shown).

**Cyclooxygenase Activity Assay** Cyclooxygenase activity was measured at 37°C by monitoring oxygen consumption in a 500-μl incubation cell connected to a Clark electrode (DW1 electrode unit and CB1.D oxygen control box, Hansatech Instruments, Norfolk, UK). For measurement of oxygen consumption in the presence of minoxidil, the oxygraph cell chamber was filled with 424 μl of 0.1 M Tris-HCl buffer (pH 8.00) containing 5 mM EDTA and various concentrations of minoxidil (0 μM-425 μM). Then 100 nM of PGHS-1 (from a stock solution, 0.235 mg of 82,000 units mg/ml) without phenol and exogenous hemin was added and after 1 min of incubation, the reaction was started by the addition of 10 μl of 6.7 mM potassium arachidonate (final concentration, 150 μM). The oxygen consumption was calculated with the assumption that at 37°C, 260 μM of $O_2$ was dissolved as described (Nastainczyk *et al*, 1984).

**High Pressure-Liquid Chromatography/Enzymatic Immunoassay (EIA) Analysis** Three individual and distinct experimental reactions between PGHS-1 (170 nM), minoxidil (500 μM), and arachidonic acid (150 μM) in a total volume of 500 μl were monitored for oxygen consumption at 37°C. Samples (50 μl) were then taken after completion of the reaction (as evidenced by the absence of oxygen uptake), kept for 30 min at room temperature, and then stored at −80°C. It was shown that $PGH_2$, which is unstable under these conditions, formed stable compounds (respectively $PGE_2$ and 15 hydroperoxy-$PGE_2$) by nonenzymatic decomposition. The yield of this spontaneous decomposition was about 90%, with 10% of $PGH_2$ being converted to $PGD_2$ (Eling *et al*, 1991). To measure $PGE_2$ formation and avoid any interference with arachidonic acid in the EIA system, we performed an extraction prior to HPLC purification based on a method developed by Kelly *et al* (1989). Briefly, each sample was acidified with 20 μl of glacial acetic acid and centrifuged at 2500 × *g* for 5 min. Supernatant was removed and applied to a 100-mg $C_{18}$ Bakerbond column (Amersham, les Ulis, France). The column was then washed with 1 vol of distilled water and 1 vol of hexane. Prostaglandins were eluted with two-column volumes of ethyl acetate. Each fraction was evaporated under nitrogen, and the dry residues were dissolved in 500 μl of methanol.

**HPLC** The HPLC system (600S controller, 616 pump, 996 photodiode array detector, and Millenium software) was from Waters Associates (Milford, MA). Each fraction containing $PGE_2$ was collected after HPLC separation using a Waters Nova-Pak $C_{18}$ 4-μm (3.9 × 150 mm) column (Waters Associates). The mobile phase was methanol/water/trifluoroacetic acid (70:30:0.1) for the first 4 min, followed by a linear gradient of methanol/trifluoroacetic acid (100:0.1), which was maintained for 10 min at a flow rate of 1 ml/min. Under these conditions, $PGE_2$ and arachidonic acid standards were detected at an elution time of 2.9 min and 11.4 min, respectively. Because $PGE_2$ generated *in vitro* was below the HPLC detection level, the undetectable $PGE_2$ sample fractions were thus collected at 2.9 min and evaporated using a vacuum device from Heto (Allerod, Denmark). The dry residues were dissolved in 100 μl of EIA buffer, and their $PGE_2$ content was assayed using a more sensitive EIA system Biotrak from Amersham International (Buckinghamshire, England).

**Cell Culture** Fresh samples of human scalp skin were rapidly dissected as previously described (Philpott *et al*, 1990) under a stereo dissecting microscope using a scalpel blade and watchmaker's forceps. Individual isolated follicle units were placed in 100-mm culture dishes (Corning Glass Works, Corning, NY). Dermal papillae were microdissected as described (Messenger, 1984) and cultured in Medium 199 containing 2 mM L-glutamine, 1% of an antibiotic/antimycotic solution, and 10% fetal bovine serum (GIBCO) at 37°C, 5% $CO_2$. At the time of the first passage the dermal papilla fibroblasts were seeded in 24-well plates from Costar (Cambridge, MA) at a plating density of $5 \times 10^3$ cells/cm$^2$. The cells were cultured for 16 h in 10% fetal calf serum-supplemented Medium 199. Next, 100 μM minoxidil was added to the culture medium, and the cells were further incubated 16 h at 37°C. Minoxidil was dissolved in (20:80) ethanol/water, the final concentration of ethanol being <1%. $PGE_2$ amounts were directly estimated using a Biotrak $PGE_2$ EIA system (Amersham International, Buckinghamshire, England). BALB/c 3T3 fibroblasts (clone A31) were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were seeded into 24-well plates at a plating density of $10^3$ cells/cm$^2$. The cells were cultured for 16 h in 10% fetal calf serum-supplemented Medium 199. Minoxidil (100 μM) was added to the culture medium and $PGE_2$ production measured under the same conditions as we used for dermal papilla fibroblasts.

## RESULTS

**Localization of PGHS-1 and PGHS-2 Isoforms in Human Hair Follicles** Using specific MoAbs, we first determined which PGH-S isoforms were expressed in the human hair follicle and in which hair follicle compartment. As shown in **Fig 1**, we observed that PGHS-1 was produced in the lower part of the hair follicle. PGHS-1 protein was strongly (and preferentially) expressed in the dermal papilla cells during anagen **(Fig 1*a*)** and late catagen phase **(Fig 1*b*)**, suggesting that PGHS-1 is present in these cells throughout the hair cycle. In contrast, labeling with MoAb against PGHS-2 showed very weak staining of anagen dermal papilla cells **(Fig 1*c*)** and no staining of late catagen dermal papilla cells **(Fig 1*d*)**. In addition, strong expression of PGHS-1 was detected in the perifollicular epidermis **(Fig 1*e*)** in the absence of expression of PGHS-2 **(Fig 1*f*)**. These results indicate that PGHS-1 is the main isoform expressed in the hair follicle and in the perifollicular epidermis and additionally they show that its location is restricted to the dermal papilla during the human hair cycle.

**Concentration-Dependent Enzymatic Activation of PGHS-1 by Minoxidil *In Vitro*** Because PGHS-1 is the major isoform in human hair follicle, and taking into account that based on 91% protein sequence homology of human and sheep PGHS-1 (Smith and Marnett, 1991) and that sheep PGHS-1 is widely used as model for human PGHS-1 in mechanistic studies, we investigated the sensitivity of sheep PGHS-1 to minoxidil. As shown in **Fig 2**, PGHS-1 is strongly activated by minoxidil. The activation of PGHS-1 by minoxidil is evidenced both by an increase in the slope, indicating an acceleration of the catalytic process, as well as by an increase of the total $O_2$ consumption compared with the control, (arachidonic acid alone). Moreover, as shown in **Table I**, this activation is concentration-dependent, and the AC50 for PGHS-1 activation was 80 μM. As expected, the PGHS inhibitor indomethacin completely inhibited the PGHS-1 activity **(Table II)**. The calculated total amounts of $O_2$ consumed under the different

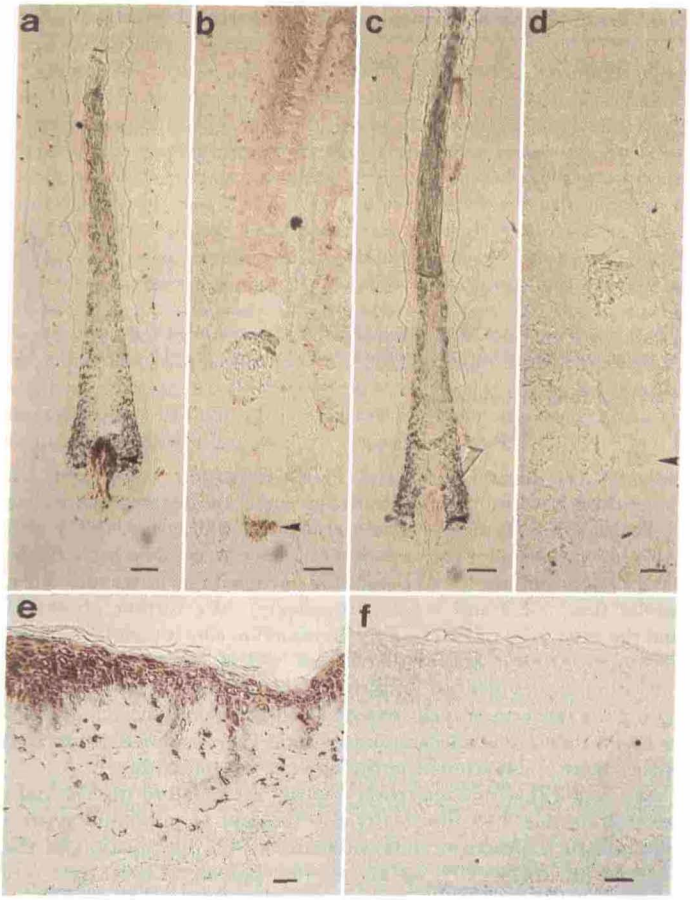


**Figure 1. PGHS-1 is the major PGHS expressed in dermal papilla from anagen and catagen human hair follicles.** Longitudinal section of hair follicles were incubated with either anti-PGHS-1 *(a,b)* or anti-PGHS-2 *(c,d)* MoAbs for 18 h at 4°C prior to peroxidase staining as indicated in *Materials and Methods*. Anagen hair follicle *(a,c)*. Catagen hair follicle *(b,d)*. Note the weak expression of PGHS-2 in dermal papilla from anagen hair follicle and the lack of expression of PGHS-2 in dermal papilla from catagen hair follicle *(d)*. Control experiment showing the expression of PGHS-1 *(e)* but not of PGHS-2 *(f)* in the epidermis. *Scale bar*, 50 μm. ➤, dermal papilla

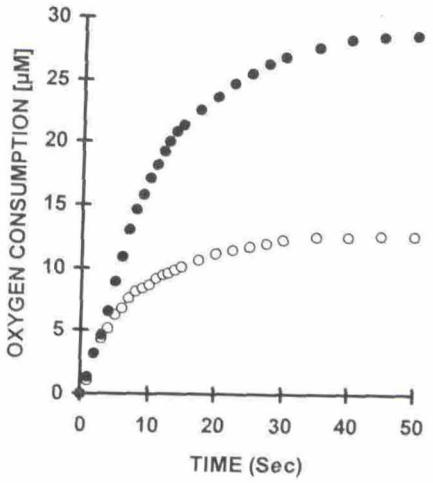


**Figure 2. Enzymatic activation of purified PGHS-1 *in vitro* by minoxidil.** Oxygen consumption by purified ovine PGHS-1 (E.C. 1.14.99.1) was monitored at 37°C with a calibrated Clark electrode as indicated in *Materials and Methods* (results from one representative experiment). At t = 0, 10 μl of substrate for PGHS-1 (arachidonic acid at a final concentration of 150 μM) were added and changes in oxygen consumption were immediatly recorded for 50 s. (-○-) Control without minoxidil; (-●-) 500 μM minoxidil.

stimulation conditions (see **Table I** and **Table II**) indicate that the oxygen uptake in response to minoxidil can be increased up to 2-fold compared with the control. Furthermore, a similar increase (×1.53) in the amount of $PGE_2$ was generated *in vitro* by the purified PGHS-1 in response to minoxidil (see **Table II**).

**Induction of $PGE_2$ Minoxidil Production in the Supernatant of Dermal Papilla Cells and BALB/c 3T3 Fibroblasts in Culture *In Vitro*** To ascertain that the PGHS-1 activation by minoxidil demonstrated at the enzymatic level was biologically significant, the production of $PGE_2$ by living dermal papilla cells and BALB/c 3T3 fibroblasts was also measured in response to minoxidil. **Fig 3** shows that 100 μM minoxidil induced production of $PGE_2$ in the supernatant of human dermal papilla fibroblasts **(Fig 3*a*)** as well as in that of BALB/c 3T3 fibroblasts in culture *in vitro* **(Fig 3*b*)**. $PGE_2$ production (pg/ml) was significantly increased ($p < 0.05$, n = 10, unilateral t test), up to 17% by dermal papilla fibroblasts treated with minoxidil compared without control untreated cells (from 75 ± 13 to 88 ± 20, mean ± SD). In BALB/c 3T3 fibroblasts $PGE_2$ production was also significantly increased ($p < 0.05$, n = 4, t test), up to 28% after minoxidil treatment (from 107 ± 2 to 137 ± 10, mean ± SD). At this concentration (100 μM), minoxidil had no effect on the proliferation rate of the treated cells (data not shown). In control experiments, as expected, 50 μM indomethacin strongly inhibited (−95%) $PGE_2$ production by BALB/c 3T3 ($p < 0.01$, t test, n = 6) as reported by Danesch *et al*, 1994. Additionally, 20 μM indomethacin also significantly, but to a lesser extent (i.e., −16%) ($p < 0.01$, t test, n = 10), inhibited $PGE_2$ production by dermal papilla cells in culture *in vitro* (data not shown).

## DISCUSSION

PGHS-1 exhibits two distinct and complementary enzymatic activities: a cyclooxygenase activity that transforms arachidonic acid into $PGG_2$ and a peroxidase activity that reduces $PGG_2$ to $PGH_2$ (Smith and Marnett). $PGH_2$ is then converted, depending on cells or tissue type–specific isomerases, to a combination of prostanoids ($PGE_2$, $PGD_2$, $PGF2\alpha$, $PGI_2$, and $TXA_2$) that have important and pleiotropic biologic effects, usually in an autocrine or paracrine manner (DeWitt, 1991; Smith and Marnett, 1991).

Two PGHS isoenzymes have been characterized. The first isoform (PGHS-1), initially purified and cloned from sheep vesicular glands (Miyamoto *et al*, 1976; Van der Ouderaa *et al*, 1977), is a homodimer of 70 kDa constitutively expressed in most tissues and very well conserved among species. Complementary DNA for sheep, mouse, and human PGHS have been cloned (DeWitt and Smith, 1988; Merlie *et al*, 1988; Yokoyama *et al*, 1988; Yokoyama and Tanabe, 1989) and amino acid sequences of the PGHS-proteins deduced. The results showed 88% sequence identity between the sheep and the mouse enzyme and 91% between the sheep and the human enzyme. Although PGHS-1 is essential for the homeostasis of mammalian cells (Vane and Botting, 1995), the second isoform PGHS-2, which presents 60% homology with PGHS-1, is reported to be inducible.

Pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor-α as well as growth factors such as epidermal growth factor are transcriptional activators of PGHS-2 (Xie *et al*, 1992). Consequently, PGHS-2 is the target of choice for nonsteroidal anti-inflammatory drugs. By contrast, inhibition of the cytoprotective enzyme PGHS-1 is detrimental to cytoprotection (as evidenced by the well-known deleterious effects of the nonselective nonsteroidal anti-inflammatory drugs, aspirin and indomethacin, on gastric cells) (Battistini *et al*, 1994; Vane and Botting, 1995). Despite being strongly expressed both at the mRNA and protein levels in a control experiment (i.e., dermal papilla (DP) cells in culture *in vitro*

**Table I. Concentration-Dependent Activation by Minoxidil of Total Oxygen Consumption by Purified PGHS-1[a]**

| | Total $O_2$ uptake ($\mu$M) | | | $V_{max}$ (katal (mol/s) $\times 10^3$) | | |
|---|---|---|---|---|---|---|
| Minoxidil ($\mu$M) | Exp. 1 | Exp. 2 | Control[b] (%) | Exp. 1 | Exp. 2 | Control[b] (%) |
| 425 | 34.8 | 34.8 | 210 | 3.02 | 3.02 | 215 |
| 215 | 27.0 | 34.3 | 185 | 2.54 | 2.70 | 188 |
| 100 | 25.0 | 26.5 | 155 | 2.28 | 2.34 | 165 |
| 45 | 21.7 | 21.7 | 131 | 1.97 | 1.72 | 132 |
| 20 | 21.8 | 20.8 | 128 | 1.87 | 1.77 | 130 |
| 0 | 16.1 | 17.2 | 100 | 1.30 | 1.51 | 100 |

[a] Oxygen consumption by purified ovine PGHS-1 from sheep seminal vesicles (E.C. 1.14.99.1) was monitored at 37°C with a calibrated Clark electrode in the presence of substrate for PGHS-1 (arachidonic acid at a final concentration of 150 $\mu$M) and increasing concentration (0-425 $\mu$M) of minoxidil as indicated in *Materials and Methods*. Total $O_2$ uptake was recorded at 1 min after the beginning of each reaction.

[b] Percent (%) of control is calculated from the mean of each group of values. AC50 = 80 $\mu$M (representative experiment).

stimulated with interleukin-1$\beta$ 50 ng/ml; data not shown), PGHS-2 isoform could be only faintly detected in normal anagen DP cells. This suggests that the PGHS-1 isoform is the major source of prostaglandin production in the DP of both anagen and catagen human hair follicle. Recent data on the PGHS-1 knock-out mouse, however, support the hypothesis that PGHS-2 could also contribute to the basal level of prostaglandin production necessary for normal homeostasis (Langenbach *et al*, 1995; Morham *et al*, 1995). Thus, because we observed that minoxidil (500 $\mu$M) could also significantly (+160%, $p < 0.05$, t test, $n = 3$) activate purified sheep placental PGHS-2 (E.C.1.14.99.2) *in vitro* (data not shown), it is not totally excluded that in normal anagen, not only PGHS-1 but also PGHS-2 might be the target of minoxidil action. By contrast, the faint expression of PGHS-2 observed in anagen dermal papillae was lost in catagen DP cells. Whether this extinction of PGHS-2 in catagen DP cells is the cause or a consequence of the transition from anagen to catagen remains to be established.

During the metabolism of arachidonic acid by the PGHS-1, many xenobiotics, such as aromatic amines or phenol, may act either as co-substrates for the peroxidase activity or may reduce the intermediate iron-oxo species into reduced iron (III)-PGHS, by two one-electron transfer reactions, leading to the protection (against "suicide" inactivation) and activation of PGHS-1 as evidenced by enhanced oxygen uptake necessary for the cyclo-oxygenation of arachidonic acid by purified PGHS-1 *in vitro* (Markey *et al*, 1987; Eling *et al*, 1990; Mahy *et al*, 1993). We found that the hair growth-promoting drug minoxidil, which could be considered as an aromatic amine, activated PGHS-1 activity *in vitro*. The expected metabolite of this reaction is prostaglandin $H_2$ ($PGH_2$), which spontaneously decomposes into $PGE_2$ and 15 hydroperoxy-$PGE_2$ *in vitro* (Eling *et al*, 1991). Using a two-step procedure (i.e., HPLC purification followed by EIA determination), we measured the increase (+53%) in $PGE_2$ synthesis by purified PGHS-1 as a result of this enzymatic activation *in vitro*. A stoichiometric relation between $O_2$ consumption and $PGE_2$ formation could not be established because the spontaneous yield of decomposition of $PGH_2$ into $PGE_2$ *in vitro* is not clearly known (Eling *et al*, 1991) and probably varies under the experimental conditions. The increase in basal $O_2$ consumption and basal $PGE_2$ production, however, were similar (i.e., $\times 2.2$ and $\times 1.5$, respectively). We further observed that the activation of PGHS-1 by minoxidil *in vitro* is concentration-dependent in the micromolar range (AC50 = 80 $\mu$M). This activation was evidenced both by acceleration of $O_2$ consumption during the reaction of cyclo-oxygenation of arachidonic acid as well as by an increase in the amount of total $O_2$ consumed compared with a control experiment in the absence of minoxidil.

We found that this activation was not restricted to the DP cells because murine 3T3 fibroblasts also respond to minoxidil with a significant (+ 28%; $p < 0.05$) increase in $PGE_2$ production in the supernatant (from 107 pg/ml to 137 pg/ml). These data are consistent with observations by others indicating that DP fibroblasts grown *in vitro* respond to minoxidil by producing at least $PGE_2$ (Lachgar *et al*, 1996). It is noteworthy that the effects observed in this study, as well as most of the biologic and chemical effects of minoxidil reported *in vitro*, such as inhibition of lysyl hydroxylase (Murad *et al*, 1992), inhibition of keratinocyte proliferation (O'Keefe and Payne, 1991), and inhibition of prostacyclin synthase (Kvedar *et al*, 1988), occur at concentrations higher (in the 25–1000 $\mu$M range) than those achieved in serum after either topical or oral administration of the drug (0.02 $\mu$M to 0.7 $\mu$M, respectively) (Orfanos, 1990). Because an accumulation of minoxidil in the epithelial matrix cells of hair follicles just above Auber's critical line (i.e., adjacent to the dermal papilla) has also been described after topical application (Buhl *et al*, 1992b), this apparent discrepancy may be related to tissue concentration of the drug *in vivo* in

**Table II. Minoxidil Induces Oxygen Consumption and $PGE_2$ Production by Purified PGHS-1 *In Vitro*[a]**

| | Control | Minoxidil (500 $\mu$M) | Indomethacin (100 $\mu$M) |
|---|---|---|---|
| Total oxygen uptake ($\mu$M) | 12 ($\pm$0.7) | 27[b] ($\pm$1.3) | 0[b] |
| Control (%) | 100 | 225 | 0 |
| PGE2 ($\mu$M) | 1.5 ($\pm$0.3) | 2.3[b] ($\pm$0.15) | 0[b] |
| Control (%) | 100 | 153 | 0 |

[a] According to the description by Eling (Eling *et al*, 1991). The reaction medium from the Clark electrode cell chamber after completion of the *in vitro* reaction was evaluated for $PGE_2$ content using an enzyme immunoassay (EIA) as described in *Materials and Methods* after HPLC purification. Arachidonic acid 150 $\mu$M. Mean $\pm$ SD, $n = 3$.

[b] Analysis of variance; $p < 0.05$. Ovine PGHS-1 was isolated from sheep seminal vesicles. (EC 1.14.99.1).

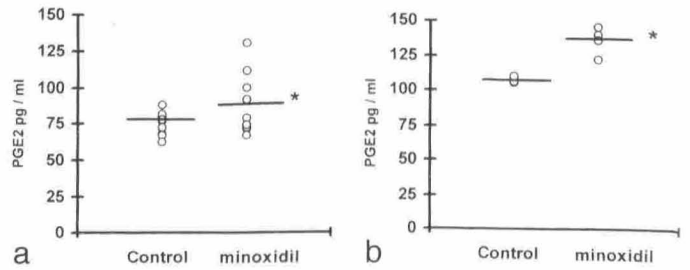


**Figure 3. Induction of $PGE_2$ production in response to minoxidil by fibroblasts in culture *in vitro*.** $PGE_2$ production levels in supernatants from either control or 16-h minoxidil-treated cells (100 $\mu$M) were evaluated using an EIA as described in *Materials and Methods*. Supernatant from dermal papilla fibroblasts of human hair follicles in culture *in vitro* (mean values from four wells) *(a)*. Supernatant from BALB/c 3T3 fibroblasts in culture (mean values from 10 wells) *(b)*. * Indicates results that differ significantly from control ($p < 0.05$, unilateral t test).

proximity to the dermal papilla in which PGHS-1 activation or protection might occur.

In humans, Olsen and Delong (1990) showed no beneficial effect of the $PGE_2$ analog viprostol on androgenetic alopecia. The lack of activity of viprostol in their study might be due to either an inadequate frequency of application (twice daily) or a concentration inadequate to support sustained exposure of DP cells or adjacent matrix cells to PGs. By contrast, more recent studies (Malkinson *et al*, 1993) have shown a cytoprotective effect of $PGE_2$ against either radiation- or doxorubicin-induced hair loss *in vivo* (on mouse dorsal skin) with a maximal activity 2 h after application (and a total loss of protective activity of $PGE_2$ 4 h after application). Furthermore, topical application of the PGHS inhibitor indomethacin was found to delay hair growth *in vivo* in mice.[1] Together, these observations strongly suggest that the cytoprotective effect of minoxidil that we describe here should be considered in the complex process by which minoxidil stimulates hair regrowth *in vivo*.

*We wish to sincerely thank Pr. Dr. Hans Schaefer and Dr. Claude Bouillon for their critical and expert comments on the manuscript.*

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