Pterins in Human Hair Follicle Cells and in the Synchronized Murine Hair Cycle

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Human dermal papilla cells (HDPC) express mRNA for the key enzymes for *de novo* synthesis/recycling and regulation of the pterin (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin ($6BH_4$). HDPC had significantly higher enzyme activities and $6BH_4$ levels in a comparative study with dermal fibroblasts, epidermal melanocytes, and keratinocytes under *in vitro* conditions. In addition, a significantly more rapid uptake of ^{14}C-L-phenylalanine was demonstrated in HDPC compared with fibroblasts, whereas the differences in turnover to L-tyrosine were insignificant, suggesting a pooling of L-phenylalanine in HDPC. These results suggested that HDPC driven $6BH_4$ synthesis could be of major functional importance in the hair cycle. In order to follow this hypothesis *in vivo*, expression of enzyme activities and levels of the produced cofactor during the synchronized hair cycle were determined employing the murine model C57BL/6. These data revealed a significantly increased *de novo* synthesis for $6BH_4$ via GTP-cyclohydrolase I concomitant with high levels of $6BH_4$, and the induction of phenylalanine hydroxylase activities during the telogen/early anagen stage (days 0–1). Pterin levels and enzyme activities fall on day 3 and plateau during the rest of the entire cycle. In addition, thioredoxin reductase and glutathione reductase activities were measured, where the latter enzyme remained constant but thioredoxin reductase activities showed a biphasic behavior. The first peak coincided with the induction of $6BH_4$ *de novo* synthesis at the beginning of the hair cycle. The second peak was observed at mid-anagen, when melanogenesis takes place. Taken together, our results show the presence of autocrine pterin synthesis/recycling in human hair follicle cells under *in vitro* conditions, and a possible role for $6BH_4$ in the synchronized murine hair cycle. *Key words: dermal papilla/hair pigmentation/melanocytes/(6R)-L-erythro-5,6,7,8-tetrahydrobiopterin. J Invest Dermatol 111:545–550, 1998*

The field of pterin and pteridine research began with their isolation from the yellow pigment in butterflies more than 100 y ago (Hopkins, 1889) and was continued by Schöpf and Reichert (1941). One of the most important compounds is (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin ($6BH_4$), functioning as an essential cofactor/electron donor for the hydroxylation of the aromatic amino acids L-phenylalanine, L-tyrosine, and L-tryptophan (Kaufman and Fisher, 1974). The importance of this cofactor was also shown in the regulation of the nitric oxide synthases (Werner *et al*, 1992). Moreover there is increasing evidence of a regulatory role for the pterins in cell-mediated immunity (Ziegler *et al*, 1990). The characteristic fluorescence of accumulated oxidized pterins in the skin of patients with vitiligo upon UVA exposure was instrumental in the recognition of these substances in the human epidermis. Since this observation, it was demonstrated that epidermal keratinocytes as well as melanocytes have the full capacity to synthesize *de novo* and recycle $6BH_4$ (Schallreuter *et al*, 1994a, b). The presence of this biosynthetic pathway enables both cells to intracellularly produce L-tyrosine from the essential amino acid L-phenylalanine via phenylalanine hydroxylase (PAH) (EC 1.14.16.1) in the presence of oxygen (Kaufman and Fisher, 1974). L-tyrosine serves as the substrate for tyrosine hydroxylase (EC 1.14.16.2), together with $6BH_4$ to produce catecholamines in keratinocytes (Schallreuter *et al*, 1992, 1995). In melanocytes this amino acid is the substrate for tyrosinase, the key enzyme for melanogenesis (Prota, 1992). Only recently a new function for $6BH_4$ was recognized by controlling the initial step of pigmentation in epidermal melanocytes (Schallreuter-Wood and Wood, 1995; Wood *et al*, 1995). A specific binding domain for the reduced cofactor on tyrosinase (EC 1.14.18.1) was identified, where the binding of $6BH_4$ leads to the inhibition of the enzyme, whilst the oxidized 6-biopterin has no effect on enzyme activities (Wood *et al*, 1995). There is strong evidence that the redox status of $6BH_4$/6-biopterin can affect melanocyte survival and pigmentation as well as the differentiation process in keratinocytes (Schallreuter *et al*, 1995). Thioredoxin/thioredoxin reductase (T/TR) (EC 1.6.4.5) can control pterin redox status where T/TR reduces 6-biopterin to quinonoid dihydropterin (qBH_2), which can be fully reduced to $6BH_4$ by either dihydropteridine reductase (EC 1.6.99.7) or reduced glutathione (Schallreuter *et al*, 1995; Wood *et al*, 1995).

The first and rate-limiting enzyme in the *de novo* synthesis of $6BH_4$ is GTP-cyclohydrolase I (GTP-CH-I) (EC 3.5.4.16) (Nichol *et al*, 1985). This enzyme can be subject to regulation by interleukin 2, interferon gamma, mast cell growth factor, and tumor necrosis factor alpha (Ziegler *et al*, 1990, 1993; Werner *et al*, 1992). The initiation of $6BH_4$ *de novo* synthesis is followed by two further enzymatic steps. The cycling of the cofactor begins with the conversion of L-phenylalanine to

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Abbreviations: GFRP, GTP-CH-I feedback regulatory protein; GTP-CH-I, GTP-cyclohydrolase I; HDPC, human dermal papilla cells; PAH, phenylalanine hydroxylase; $6BH_4$, (6R)-L-erythro 5,6,7,8 tetrahydrobiopterin; T/TR, thioredoxin/thioredoxin reductase.

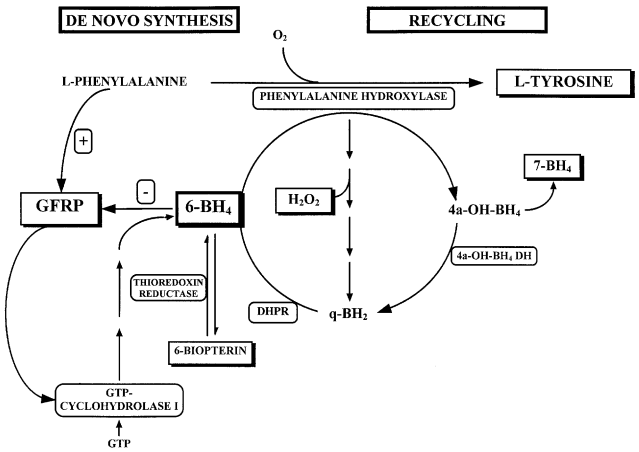

Figure 1. The *de novo* synthesis/recycling and regulation of $6BH_4$. GTP-CH-I is the rate limiting enzyme in the synthesis of $6BH_4$. This cofactor serves for the metabolism of the essential amino acid L-phenylalanine to L-tyrosine via PAH. The recycling of $6BH_4$ involves its conversion to 4a-OH-$6BH_4$ concomitant with the nonenzymatic production of low levels of the isomer $7BH_4$. 4a-OH-BH_4 is dehydrated by the rate-limiting enzyme 4a-OH-$6BH_4$-dehydratase (4a-OH-BH_4-DH) forming quinonoid dihydropterin (qBH_2), which is reduced to $6BH_4$ by dihydropteridine reductase (DHPR). The oxidation of $6BH_4$–6-biopterin can be reversed by thioredoxin reductase. The GFRP controls the *de novo* synthesis of $6BH_4$, where L-phenylalanine upregulates GTP-CH-I activities, meanwhile $6BH_4$ exerts the opposite effect.

L-tyrosine via PAH leading to 4a-carbinolamine and the nonenzymatic generation of the isomer (7R)-L-erythro-5,6,7,8-tetrahydrobiopterin ($7BH_4$). 4a-carbinolamine is metabolised further by 4a-hydroxy-tetrahydrobiopterin dehydratase (DH; synonyms: phenylalanine stimulating factor, 4a-carbinolamine dehydratase) (EC 3.4.5.10) to quinonoid dihydropterin (qBH_2) followed by its reduction through dihydropteridine reductase to $6BH_4$. The *de novo* synthesis is directly controlled by the GTP-CH-I feedback regulatory protein (GFRP) with L-phenylalanine stimulating GTP-CH-I and $6BH_4$ inhibiting the enzyme (Harada *et al*, 1993). This pathway is presented in **Fig 1**.

Recently the presence of the pterins has been demonstrated in human hair shafts but the origin of these compounds has escaped definition so far (Sawada *et al*, 1996). During the entire life time the hair follicle undergoes cyclic switches between periods of proliferation and terminal differentiation (anagen), followed by very rapid involution of the follicle (catagen) and a resting period (telogen) (Chase, 1954; Kligman, 1959; Paus, 1996). It is generally believed that the control center for this cascade resides in the dermal papilla (Oliver, 1970; Jahoda *et al*, 1984; Hardy, 1992; Paus, 1996). The formation of a pigmented growing hair is not yet completely understood. Melanogenesis has been shown to be strictly linked to the hair cycle, only occurring in anagen III–VI, whereas it ceases in catagen and is completely absent in telogen (Jahoda and Oliver, 1981; Jahoda *et al*, 1984; Ebling, 1987; Ortonne and Prota, 1993; Slominski and Paus, 1993). This process has been attributed to a concerted action between follicle melanocytes, keratinocytes, and human dermal papilla cells (HDPC) (Straile *et al*, 1961; Slominski and Paus, 1993).

Since the pterins in the epidermal unit are closely linked to catecholamine synthesis/cell differentiation in keratinocytes and melanogenesis in melanocytes, and because pterins have recently been found in the hair shaft, it was of interest to identify which cells could be candidates for the production and accumulation of this cofactor in the hair follicle (Sawada *et al*, 1996). In addition, it seemed interesting to elucidate a possible role for $6BH_4$ as a local regulator of the "the biologic clock" controlling the hair cycle. For this purpose an *in vitro* system and an *in vivo* system were investigated: (i) human follicle keratinocytes and melanocytes as well as HDPC and dermal fibroblasts were established and analyzed in cell culture; (ii) furthermore we utilized the C57BL/6 mouse as this model allows detailed studies during the synchronized hair cycle (Paus *et al*, 1990; Slominski *et al*, 1991).

MATERIALS AND METHODS

Cell cultures HDPC and human dermal fibroblasts were established in E199 or RPMI-1640 medium containing 10% (vol/vol) human serum following the method of Messenger (1984). Human epidermal undifferentiated and differentiated keratinocytes and melanocytes were established in serum free MCDB 153 medium according to the methods of Wille *et al* (1984) and Pittelkow and Shipley (1989). Human hair follicle keratinocyte and melanocyte cell cultures were established as described by Tobin *et al* (1995).

Mice Six to nine week old, syngenic, female C57 BL/6 mice in the telogen phase of the hair cycle or pregnant mothers were purchased from Charles River (Sulzfeld, Germany). The mice were housed in community cages with 12 h light periods at the Humboldt Universitat, Berlin, Virchow Klinikum. The animals were fed water and mouse chow *ad libitum*.

Hair cycle induction The hair cycle was induced by depilation in the back skin of the mice in the telogen phase as previously described (Paus *et al*, 1990; Slominski *et al*, 1991). At defined days after depilation the mice were sacrificed and their back skin was harvested and snap frozen in liquid nitrogen (Paus *et al*, 1994a, b). The precise stage of the hair cycle was confirmed by ultrastructural analyses employing conventional electron microscopy as previously described (Tobin *et al*, 1993).

Preparation of cell extracts Cytosolic cell extracts were prepared from monolayers of cultured cells or from total skin of mice according to the method of Schallreuter *et al* (1991). Protein concentration was estimated using the method of Kalb and Bernlohr (1977).

Determination of total epidermal biopterins by high performance liquid chromatography Biopterins were analyzed after acidic iodine oxidation and prepurification by cation exchange chromatography using DOWEX AG 50 (Wx8) followed by reverse phase high performance liquid chromatography and fluorimetric detection as previously described (Kerler *et al*, 1990; Ziegler and Hültner, 1992). Biopterin levels were determined as pmoles per mg protein.

Enzyme assays GTP-CH-I activity was determined by reverse phase high performance liquid chromatography following the formation of neopterin with alkaline phosphatase using the methods of Kerler *et al* (1990) and Blau and Niederwieser (1983). Specific activity of GTP-CH-I was defined as pmoles neopterin mg per protein per h.

PAH activity was evaluated following the formation of [^{14}C] labeled tyrosine from [^{14}C] U-L-phenylalanine, using the method of Schallreuter *et al* (1994a, b). Activities were determined in nmoles per mg protein per min.

Thioredoxin reductase (TR) and glutathione reductase (GR) (EC 1.6.4.2) activities were measured in cell extracts following the reduction of 5,5-dithiobis (2-nitrobenzoate) at 412 nm ($\Delta\varepsilon_{412}$ = 13600, pH 7–8) following a modified method of Schallreuter and Wood (1986). Briefly, for evaluation of combined enzyme activities (TR/GR) the reaction mixture contained Tris buffer (0.5 M pH 7.5), bovine serum albumin (10 mg per ml), ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′,-tetraacetic acid (2 mM), NADPH (25 mM), and 5,5-dithiobis (2-nitrobenzoate) (5 mM). The reaction was started at 37°C after the addition of cell extract. Each sample was incubated for 1 h.

GR activities were analyzed separately in identical reaction mixtures without the addition of ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′,-tetraacetic acid, where TR activities were completely inhibited with calcium chloride (50 mM) (Schallreuter and Wood, 1986). TR activities were calculated by subtraction of GR activity from the combined enzyme activities.

Transport of ^{14}C-phenylalanine and its turnover to L-tyrosine in HDPC and fibroblasts ^{14}C-L-phenylalanine uptake was followed over 20 min in intact confluent cell cultures of HDPC and dermal fibroblasts. Briefly, ^{14}C-L-phenylalanine (10^{-4} M) was added to the culture medium. At different time intervals (1, 2, 5, 10, 20 min) the medium was drained, followed by a three times wash with phosphate-buffered saline (pH 7.0), followed by trypsination (1 mg per ml). The cell extracts were applied to cellulose thin layer plates and cochromatographed with a phenylalanine/tyrosine standard in a solvent containing chloroform:methanol:water:ammonium hydroxide (29:15:1:4 vol/vol). The aromatic amino acids were visualized with ninhydrin. The spots were removed and counted in scintillation fluid in the ^{14}C channel using a Beckman Scintillation counter. Radioactivity was standardized to 1 mg protein and converted into 10^{-6} M to compare intracellular concentrations of the amino acids.

Expression of epidermal and hair follicle cell mRNA using reverse transcriptase-polymerase chain reaction (PCR) Total RNA was extracted

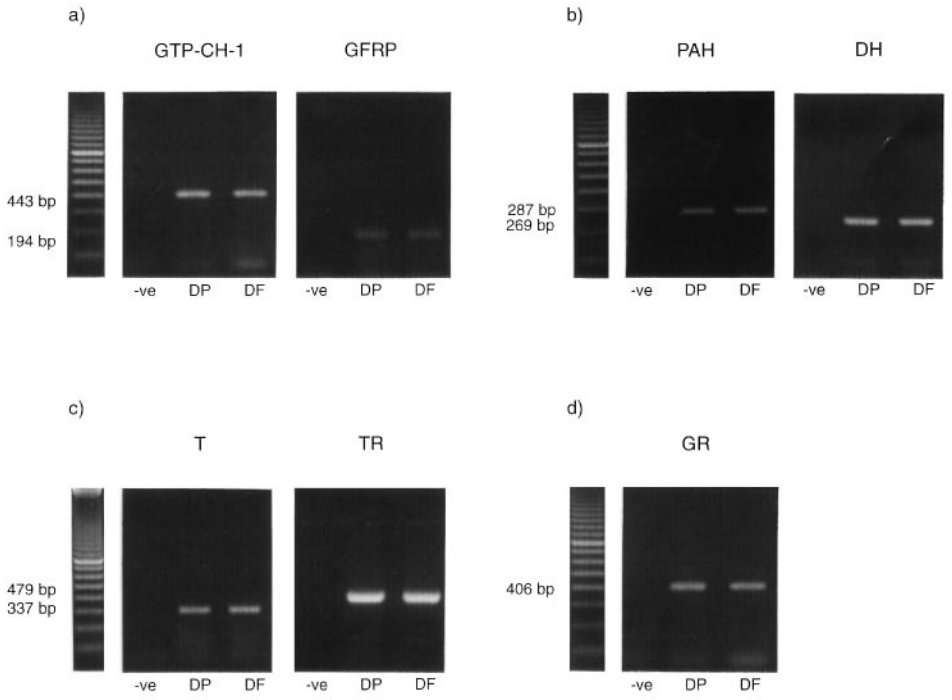

Figure 2. Reverse transcriptase-PCR analyses of mRNA expression in HDPC and human dermal fibroblasts compared with negative controls. (*a*) GTP-CH-I, GFRP; (*b*) PAH, DH; (*c*) T/TR; (*d*) GR.

Table I. Specific activities of GTP-CH-I, PAH, TR, and GR in different human skin cells. Results are the mean of at least two experiments

	Dermal papilla	Dermal fibroblasts	Epidermal melanocytes	Epidermal keratinocytes	
				undifferentiated	differentiated
GTP-CH-I (pmoles neopterin per mg protein per h)	4.28	0.94	3.3	0.17	0.01
PAH (nmoles per mg per min)	0.78	0.33	3.94	6.99	0.225
TR (nmoles per mg per h)	2.2	2.2	8.0	8.5	3.8
GR (nmoles per mg per h)	15.4	10.0	17.0	63.0	27.2

Table II. Comparison of 6- and 7-biopterin levels in different human skin cells. Results are the mean of at least three experiments

	Dermal papilla	Dermal fibroblasts	Epidermal melanocytes	Epidermal keratinocytes[a]	
pmoles per mg protein				undifferentiated	differentiated
6-Biopterin	25.14	2.85	17.20	5.97	2.50
7-Biopterin	2.78	0.30	8.30	ND	ND

[a]ND, below limit of detection.

from cell cultures using Ambion Totally RNA Isolation Kit (AMS Biotechnology, Oxon, U.K.) and subjected to reverse transcriptase-PCR analysis as described previously (Schallreuter *et al*, 1998). Primers and conditions for the PCR reactions were also as described by Schallreuter *et al* (1998) except for thioredoxin reductase and thioredoxin, which are described below. All PCR products were separated on a 1.5% agarose gel.

Thioredoxin reductase (TR) Primers designed were based upon the cDNA sequence (Genebank accession number G29720): 5′ACATGGAAGAACATGGCA3′ and 5′CTCCTCAGAAAGGCCACAAG3′. PCR conditions were 35 cycles at 92°C 1′, 60°C 1′, and 72°C 1′.

Thioredoxin (T) Primers designed were based upon the cDNA sequence (Genebank accession number J04026): 5′TTTCCATCGGTCCTTACAGC3′ and 5′TTGGCTCCAGAAAATTCACC3′. PCR conditions were 35 cycles at 35°C 1′, 60°C 1′, and 72°C 1′.

Chemicals L-[U-^{14}C]-phenylalanine (513 mCi per mmol) was purchased from Amersham Life Sciences (Little Chalfont, U.K.). $6BH_4$, $7BH_4$, 6-biopterin, and 7-biopterin were from Dr. Schircks (Jona, Switzerland). All other reagents were from Sigma (Poole, Dorset, U.K.).

Statistical analyses Statistical analyses were carried out using the Student's paired *t* test on SPSS for Windows.

RESULTS

$6BH_4$ biosynthesis-related mRNA is expressed in HDPC Both human epidermal keratinocytes and melanocytes as well as dermal fibroblasts (Schallreuter *et al*, unpublished observation) express all of the mRNA for the *de novo* synthesis/recycling of $6BH_4$ and its regulation by GFRP (Schallreuter *et al*, 1998). Furthermore, the T/TR system for the maintenance of the redox status of this cofactor/

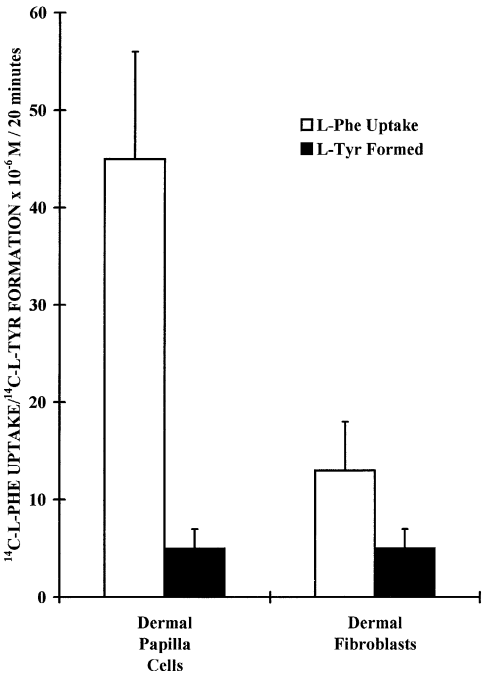

Figure 3. Intracellular ^{14}C-L-phenylalanine uptake and its turnover to ^{14}C-L-tyrosine in cell cultures. A comparative study of the intracellular ^{14}C-L-phenylalanine uptake and its turnover to ^{14}C-L-tyrosine in cell cultures of HDPC (n = 4) and human dermal fibroblasts (n = 2). The uptake and the conversion are presented as a mean ± SD.

electron donor is also expressed in these cells. In this study we showed mRNA expression and compared enzyme activities of the above systems in HDPC (n = 4) and dermal fibroblasts (n = 4). **Figure 2**(*a*) presents mRNA expression of GTP-CH-I and its regulatory protein, GFRP, the major controlling factors in $6BH_4$ *de novo* synthesis. **Figure 2**(*b*) shows the expression of the rate limiting enzymes of the $6BH_4$ recycling process, i.e., phenylalanine hydroxylase and 4a-OH-tetrahydrobiopterin dehydratase. In addition the expression of T/TR and GR is shown in **Fig 2**(*c*, *d*).

Enzyme activities and pterins are highest in HDPC Enzyme activities for GTP-CH-I, PAH, TR, and GR were determined in HDPC and dermal fibroblasts and compared with activities in epidermal melanocytes and keratinocytes. Surprisingly, the results demonstrated the highest enzyme activities for GTP-CH-I and PAH in HDPC (**Table I**). There is a significant difference between fibroblasts and HDPC ($p = 0.0001$). In order to address the importance of intracellular PAH activity we utilized high specific activity ^{14}C-L-phenylalanine and followed the uptake of this essential amino acid as well as its conversion to L-tyrosine over time in confluent cell cultures of HDPC and fibroblasts. The results yielded a significantly more rapid transport of L-phenylalanine in HDPC compared with fibroblasts under the same experimental conditions; however, the turnover to L-tyrosine was not significantly different (**Fig 3**). These results suggest that HDPC could have the capacity to pool L-phenylalanine in the 40–50 × 10^{-6} M range.

In parallel with enhanced GTP-CH-I and PAH activities the levels for the 6-biopterin were significantly higher in HDPC compared with all other cell types, whereas levels of 7-biopterin were negligible (**Table II**). Under *in vitro* conditions there were no remarkable enzyme activities for TR and GR. Taken together, the results on human cells suggested an important functional role for $6BH_4$ in the hair follicle, particularly in the dermal papilla.

Pterin synthesis is induced at the beginning of the murine hair cycle Since the human hair cycle is not synchronized, the C57BL/6 murine system was utilized to address the fate of enzyme activities and pterins during a synchronized hair cycle (Paus *et al*, 1990; Slominski

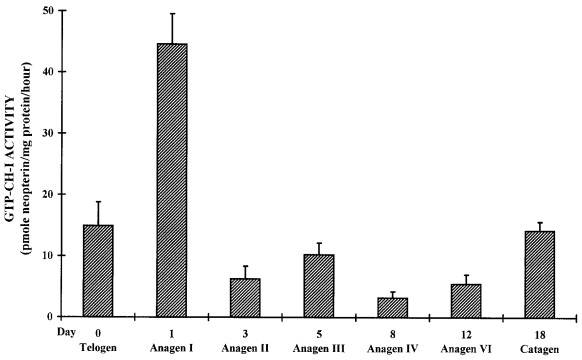

Figure 4. Specific activities of GTP-CH-I during the murine synchronized hair cycle. Specific activities of GTP-CH-I during the synchronized murine hair cycle using the C57BL/6 mouse showing a significant increase at very early anagen (day 1) (mean of four determinations ± SD).

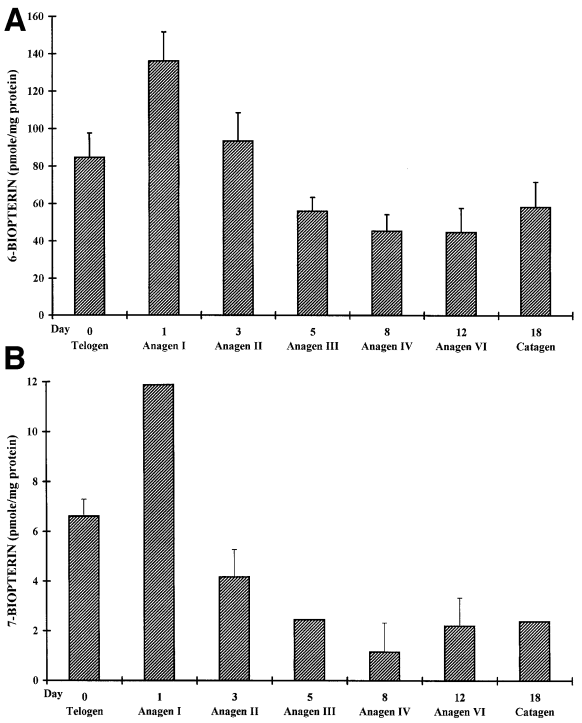

Figure 5. Total bioptein levels during the murine synchronized hair cycle. (*A*) Total 6-biopterin levels during the synchronized hair cycle in the C57BL/6 mouse yielding a peak at the early anagen (day 1) (mean of three determinations ± SD). (*B*) 7-biopterin levels during the murine hair cycle follow the same pattern as 6-biopterin, although the concentration is significantly lower and is bordering on the limit of detection. The results represent the mean of two analyses ± SD except days 1, 5, and 18, which are based on one determination.

et al, 1991). Samples were analyzed during the entire cycle (i.e., day 0, telogen; day 1, anagen I; days 3, 5, 8, 12, anagen and day 18, catagen). **Figure 4** demonstrates GTP-CH-I activities during the hair cycle. Significantly higher levels were observed in the end of telogen and in the beginning of anagen, falling to constitutive levels by day 3. Total biopterins followed a similar course with extremely high levels in the telogen/anagen stage. The nonenzymatic production of 7-biopterin followed the same pattern, although the levels were at least 20-fold lower (**Fig 5***A*, ***B***). The expression of PAH activities correlated with the time profile of GTP-CH-I and biopterin levels (**Fig 6**). The latter result would support increased $6BH_4$/PAH driven turnover of the essential amino acid L-phenylalanine to L-tyrosine during the hair cycle. A comparison of TR and GR activities showed constitutive levels for GR throughout the entire cycle, although there may be a small increase in enzyme activity in anagen at day 1. GR activities

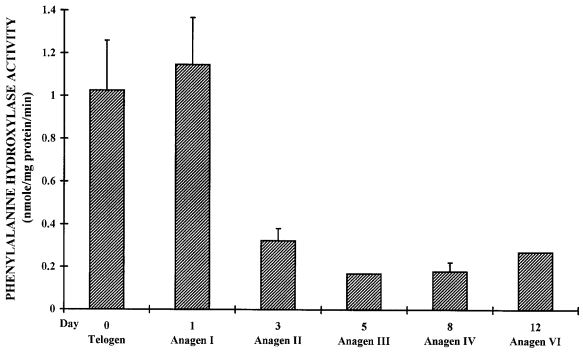

Figure 6. Specific activities of phenylalanine hydroxylase during the synchronized murine hair cycle reveal peak activities at day 0 and 1. The results show the mean of two or three experiments, except for days 5 and 12 due to a shortage of tissue extract ± SD.

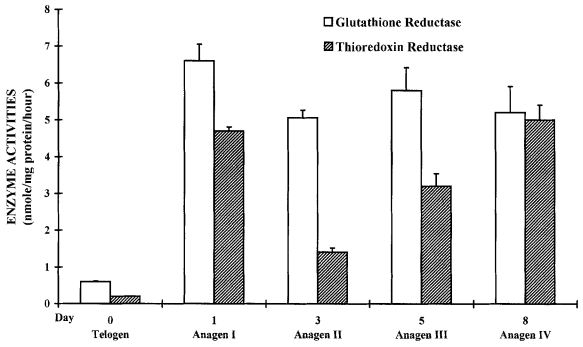

Figure 7. Specific activities of thioredoxin reductase and glutathione reductase during the murine synchronized hair cycle. A comparison of specific activities of thioredoxin reductase and glutathione reductase demonstrates constitutive activities for GR throughout the entire hair cycle, but TR activities are biphasic, peaking at day 1 and in mid-anagen at day 8 with no detectable TR after day 8 (mean of two or three experiments ± SD).

were significantly higher than TR activities; however, TR showed a biphasic induction during the hair cycle. Significantly increased TR activities were observed in anagen at day 1, falling at day 3, followed by a second induction at mid-anagen (days 5 and day 8), decreasing again in late anagen (day 12) (**Fig 7**).

DISCUSSION

The cofactor $6BH_4$ has been shown to be actively involved in the process of keratinocyte proliferation/differentiation and melanogenesis in melanocytes (Schallreuter *et al*, 1994a, 1995; Schallreuter, 1997). The discovery of pterins in the hair shaft strongly suggested the production in the hair follicle itself, and that it could be important in both hair pigmentation and regulation of the hair cycle. Here, we show that human HDPC express the full capacity for *de novo* production and recycling of $6BH_4$ under *in vitro* conditions. Interestingly, these cells produce significantly higher levels of this cofactor compared with dermal fibroblasts, and their L-phenylalanine uptake is significantly faster. These results strongly suggested a pivotal function for $6BH_4$ in these cells and possibly in the hair cycle. *In vivo* studies on the synchronized hair cycle in the C57BL/6 murine model have further explored this hypothesis. From this experimental approach several important observations have been forthcoming. There is induced $6BH_4$ *de novo* synthesis in association with high GTP-CH-I activities and synchronous high levels of $6BH_4$ in telogen/anagen at days 0 and 1 of the hair cycle, together with an increase in PAH activities. One consequence of this scenario would be enhanced production of L-tyrosine from the essential amino acid L-phenylalanine in the early anagen hair follicle. It is tempting to speculate that this L-tyrosine "pool" is a prerequisite for successful melanogenesis, because it is well established that this process requires micromolar levels of this amino acid (Wood *et al*, 1995). This assumption could be substantiated by earlier studies, where detectable expression of mRNA for tyrosinase occurred at day 2 in the murine hair cycle but enzyme activities were only clearly detectable on day 3, plateauing during mid-anagen at days 5–8 (Slominski *et al*, 1991). These results were in agreement with the studies by Sugiyama, who showed poorly differentiated, dopa-oxidase positive melanocytes in early anagen (Sugiyama, 1979). Since it has been realized that $6BH_4$ directly controls tyrosinase activities by allosteric inhibition, the low levels of $6BH_4$ between days 3–18 in the hair cycle would promote melanogenesis (Wood *et al*, 1995). On the other hand, even though tyrosinase activities would be expressed during telogen/anagen at days 0 and 1, the high $6BH_4$ levels would be sufficient to completely inhibit the enzyme (Wood *et al*, 1995). It is important to bear in mind that the actual redox status of $6BH_4$/6-biopterin will be critical to the $6BH_4$-controlled metabolic steps (Wood *et al*, 1995). One effective mechanism in the control of the $6BH_4$ redox homeostasis is the T/TR electron transfer system. The results of this study demonstrated a biphasic induction of TR during the hair cycle. The first peak of activity coincides with enhanced $6BH_4$ *de novo* synthesis and PAH activities in telogen/anagen at days 0 and 1, supporting a reduced status in the hair follicle during this period. The second peak of induced TR activities coincides with the onset of hair melanogenesis in anagen III. This induction has been observed earlier in both murine melanoma cells and brown and black guinea pig skin (Schallreuter *et al*, 1994c). It is now generally established that melanin formation also generates oxygen radicals (Menon *et al*, 1983; Zareba *et al*, 1995). Therefore it can be concluded that the induction of this very efficient antioxidant system is in response to oxidative stress (Schallreuter and Wood, 1986).

Our observations in the murine hair cycle, together with the findings reported earlier by other investigators, indicate an important role for $6BH_4$ in control of melanogenesis during the hair cycle (Slominski *et al*, 1991). If this mouse model can be extrapolated to the human hair cycle, then results from this study suggest that the dermal papilla could play an instrumental role in driving the $6BH_4$/L-phenylalanine/L-tyrosine/tyrosinase cascade for pigmentation.

Taken together, the discovery of autocrine pterin synthesis and metabolism in the hair follicle and the cycle-dependent induction of this event has opened a new view in the field of hair biology. Our future work will be directed to further investigate a possible role for $6BH_4$ in the signaling of hair growth and pigmentation.

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