Neural Mechanisms of Hair Growth Control

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Clinical and experimental observations have long suggested that skin nerves have "trophic" functions in hair follicle development, growth and/or cycling, even though the molecular and cellular basis of the underlying neuroepithelial interactions has remained obscure. Here, we critically review currently available evidence arguing in favor of or against the existence of neural mechanisms of hair growth control, and outline why the murine hair cycle provides an excellent experimental system for characterizing and manipulating piloneural interactions. Summarizing relevant, recent data from the C57BL/6 mouse model, it is pointed out that the sensory and autonomic innervation of normal pelage hair follicles, the substance P skin content, and cutaneous mast cell-nerve contacts show striking changes during synchronized hair follicle cycling. Furthermore, the murine hair follicle appears to be both a source and a target of neurotrophins, whereas neuropharmacologic manipulations alter murine hair follicle cycling *in vivo*. For example, anagen is induced by substance P or adrenocorticotropin (ACTH), and by the experimentally triggered release of neuropeptides from sensory nerves and of neurotransmitters from adrenergic nerves. Taken together, this argues in favor of neuroepithelial interactions as regulatory elements in hair growth control and suggests that the study of piloneural interactions promises important insights into general principles of neuroepithelial communication, namely during epithelial morphogenesis and remodeling. We delineate a hypothetical working model of piloneural interactions and propose that targeted manipulations deserve systematic exploration as a novel strategy for managing hair growth disorders. ***Key words: hair follicle/nerve/neuropeptides/neurotrophins/neurotransmitters. Journal of Investigative Dermatology Symposium Proceedings 2:61–68, 1997***

"TROPHIC" ROLES OF PERIPHERAL NERVES

Cutaneous nerve fibers have sensory functions, control the vasomotor tonus, and regulate the secretory activities of exocrine glands (Smith, 1996). They also exert a number of less apparent, yet important, effector functions, which include the modulation of multiple inflammatory, proliferative, and reparative cutaneous processes (Ansel *et al*, 1996). A wide array of signaling molecules released by sensory nerve fibers, and of corresponding specific receptors, is now recognized as the basis of such efferent functions of skin nerves (see this supplement).

To dermatologists, these have mostly been of interest in the context of "neurogenic inflammation," namely during hyperproliferative, inflammatory skin diseases like atopic eczema and psoriasis; in addition, neurotrophins (NTs) and neuropeptides (NPs) are increasingly appreciated as modulators of wound healing and tissue repair (Ansel *et al*, 1996; Baraniuk, 1997). The skin epithelium can also generate NTs of the nerve growth factor family [e.g., nerve growth factor (NGF), NT-3, brain-derived neurotrophic factor (BDNF), NT-4], thus influencing the development, sprouting, and survival of nerve fibers, particularly during embryonal skin development and under wound healing conditions in adult skin (Davies *et al*, 1987; Di Marco *et al*, 1991; Ernfors *et al*, 1992; Davis *et al*, 1993, 1994; Albers *et al*, 1994; Constantinou *et al*, 1994; English *et al*, 1994; Pincelli *et al*, 1994; Albers *et al*, 1996; Lewin and Barde, 1996).

This has infused new life into an ancient concept, which stipulates that peripheral nerves have a "trophic" role in epithelial tissue growth. Simple clinical observations had long suggested this: in the skin, epidermal atrophy, ulceration, and dysfunction or loss of skin appendages routinely occur as a consequence of traumatic, inflammatory, toxic, or degenerative damage to peripheral nerves (Sinclair, 1973; Walton, 1984). What are the molecular and cellular correlates of the neuroepithelial interactions in the skin, however, that underlie such "trophic" functions of peripheral nerves, and how much of these phenomena only reflect neurogenic changes in skin perfusion (see **Table I**)?

THE HAIR FOLLICLE AS A MODEL FOR STUDYING NEUROEPITHELIAL INTERACTIONS

Although this is not yet widely enough recognized, few systems can rival the hair follicle (HF) as a model for addressing these questions, and for dissecting "neurotrophic" effects on epithelial tissue growth in general. The interactions between the HF and its perifollicular neural network **(Fig 1)** offer an intriguing experimental system for the analysis and manipulation of neuroepithelial interactions during epithelial morphogenesis and remodeling under physiologic and pathologic circumstances. Furthermore, this model system allows one to explore novel neuropharmacologic strategies for the therapeutic manipulation of epithelial tissue growth in general and of hair growth in particular.

In addition, the HF itself is an exemplary epithelial-mesenchy-

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Abbreviations: BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; FNB, follicular neural network B; HF, hair follicle; MC, mast cell; NP, neuropeptide; NT, neurotrophin; NTM, neurotransmitter; p75NTR, p75 low affinity neurotrophin receptor; SP, substance P; Trk, tyrosine kinase.

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Table I. How Peripheral Nerves May Exert "Trophic" Functions on Skin Epithelium

Cutaneous nerve fibers
- → Control vasomotor tonus (thus regulating nutrient and O_2 supply to epithelium)
- → May directly alter endothelial cell functions and may stimulate angiogenesis by release of NPs like SP
- → Modulate KC functions by releasing NPs and/or NTMs, which
  a. Directly stimulate KC receptors for NPs and NTMs
  b. Indirectly affect KCs via altering the secretory activities of mesenchymal cells (e.g., MCs, macrophages, and fibroblasts)
- → May also secrete NTs

Glia cells of cutaneous nerve fibers (Schwann cells)
- → Secrete growth factors which may stimulate corresponding receptors expressed by KCs

For references, see Haegerstrand *et al*, 1989; Ziche *et al*, 1990; Rozengurt, 1991; Schallreuter *et al*, 1992, 1993; Grando *et al*, 1993; Paus *et al*, 1994c, 1995; Ansel *et al*, 1996; see also Ansel *et al*, Bothwell, Grando, Pincelli and Yaar, 1997)

mal-neuroectodermal interaction unit because it generates pigmented hair shafts as the result of tightly coordinated interactions between epithelial cells (follicle keratinocytes), specialized fibroblasts (dermal papilla cells), and neuroectodermal cells (follicle melanocytes) (Paus, 1996). The HF is the most densely innervated structure of mammalian skin, and its innervation has been extensively characterized in many functionally distinct follicle types of diverse species (cf. Winkelmann, 1960; Yamamoto *et al*, 1966; Munger and Ide, 1988; Winkelmann, 1988; Hashimoto *et al*, 1990; Ebara *et al*, 1992; Rice *et al*, 1993; Halata, 1993; Hordinsky and Ericson, 1996). Together with its accessibility, all this designates the HF an ideal study object for neurobiologic analyses.

The biologically most intriguing feature of the HF is that it spontaneously undergoes life-long, cyclic transformations from a state of relative resting (telogen) to a stage of rapid morphogenesis, intense follicle keratinocyte proliferation, and hair shaft production (anagen) **(Fig 1)**. Anagen is suddenly followed by the highly controlled regression of the proximal, cycling portion of the HF (catagen), which is largely based on keratinocyte apoptosis. This cyclic growth and regression activity is associated with significant alterations in the thickness as well as in the extracellular matrix composition and architecture of all skin compartments, most markedly in species with synchronized HF cycling (Chase, 1954; Hardy, 1992; Paus, 1996, Stenn *et al*, 1996).

On this background, we are challenged to define whether skin nerves and signaling molecules emanating from them are involved in the morphogenesis and cyclic remodeling of the HF, whether the hair cycle is associated with alterations in HF innervation, and whether the HF influences the structure and function of cutaneous nerves. In the following, this will be discussed, with a focus on potential neural mechanisms of hair growth control.

NEURAL MECHANISMS OF HAIR GROWTH CONTROL: *PRO AND CONTRA*

Every physician dealing with patients that complain of hair growth disorders sooner or later will encounter some who firmly believe that their hair loss results from chronic "stress" or a stressful life event, and the belief that "nerves" and "stress" affect hair growth remains deeply rooted in the folklore, literature, and humor of many cultures. This concept is actually as old as the beginnings of systematic hair research and is nicely illustrated by the so-called "trophoneurotic" or "psychogenic" theories on the etiology of alopecia areata, which was long believed to represent a communication disorder betwen HFs and their innervation (e.g., Joseph, 1921; cf. Simpson, 1991). In fact, the mysterious phenomenon of "overnight graying," ever so rarely seen in association with extreme psychoemotional stressors, may represent a fulminant attack of the diffuse variant of alopecia areata, which selectively attacks pigmented anagen HFs, but spares pre-existent graying or white hair (cf. Whitlock, 1976; Simpson, 1991; Paus *et al*, 1994f).

Nonetheless, despite multiple case reports in the literature that attempt to link stressful life events to the onset of telogen effluvium or alopecia areata, in our experience, such a connection can only be made in a small minority of patients with effluvium or alopecia, and very little, if any, convincing evidence has been produced to document that psychologic factors really induce hair loss (Whitlock, 1976). Overproduction of corticotropin-releasing hormone in transgenic mice, which show abnormally high ACTH and glucocorticoid serum levels and are considered a good model for chronic "stress," is indeed associated with hair loss (Stenzel-Poore *et al*, 1996). This may be related, however, to the hair growth-inhibitory effects of glucocorticosteroids (Stenn *et al*, 1993, Paus *et al*, 1994a).

As summarized in **Table II**, the currently available evidence *pro* and *contra* neural mechanisms of hair growth control is similarly ambiguous. For example, HFs can be successfully transplanted from one skin location to another, independent of their original innervation and vasculature (Unger, 1995). Transplanted HFs, however, eventually become re-innervated from the host skin site, and it is unknown whether this affects their long-term survival or cycling. HFs can also grow in organ culture, where they continue to produce a hair shaft and may even traverse a section of the hair cycle (e.g., Li *et al*, 1992b, 1992c; Philpott *et al*, 1994; Philpott and Kealey, 1994; Philpott *et al*, 1996). Nonetheless, this is limited to a rather short timespan, and progression through a full hair cycle *in vitro* has never been proved.

Likewise, even though rudimentary folliculoids can develop in

Figure 1. Schematic representation of perifollicular innervation in C57BL/6 mouse skin (modified from Botchkarev *et al*, 1997a). The figure depicts three selected hair cycle stages, showing a resting follicle (telogen), a follicle that is in the early phase of the active growth stage of the hair cycle (anagen II), and a mature anagen VI follicle, which generates a pigmented hair shaft. Note that nerve fibers in murine skin are arranged in three horizontal plexus (SEP, DCP, SCP), which feed fibers into two distinct perifollicular neural networks (FNA, FNB). The innervation details listed in the figure summarize the PGP 9.5 immunoreactivity, analyzed by confocal microscopy, in multiple skin sections (Botchkarev *et al*, 1997a). SEP, subepidermal neural plexus; DCP, deep cutaneous neural plexus; SCP, subcutaneous neural plexus; FNA, follicular network A; FNB, follicular network B; I+B, isthmus and bulge region of the ORS; SG, sebaceous gland; APM, arrector pili muscle; IRS, inner root sheath; ORS, outer root sheath; HS, hair shaft; Mel., HF melanocytes; HM, hair matrix.

the absence of skin nerves, e.g., in spheroid raft cultures of human embryonal skin (Holbrook and Minami, 1991), they never mature into hair shaft-producing and cycling HFs. Also, follicles in early stages of morphogenesis that are enzymatically dispersed from neonatal mouse skin can be transplanted into appropriate tissue beds and begin to grow largely normal hair shafts (Lichti *et al*, 1993). It is unknown, however, whether these transplanted hair pegs need to receive any NP, NT, or neurotransmitter (NTM) signals from the recipient skin in order to function normally.

Typically, damage to dorsal roots or peripheral nerves, limb paralysis, causalgia, and syringomyelia are associated with follicle atrophy in the corresponding zone of skin innervation (Sinclair, 1973). In dogs, hair growth retardation occurs after experimental sectioning of peripheral nerves and dorsal roots (Kobayashi *et al*, 1958). In rat skin, hair growth retardation and a decrease in hair shaft thickness, associated with alopecia, develop after sensory denervation by neonatal capsaicin treatment (Maggi *et al*, 1987). In neonatal mouse skin, experimental noradrenaline depletion of sympathetic nerve fibers causes localized disturbances of HF morphogenesis as well as alopecia (Asada-Kubota, 1995).

Peripheral nerve damage can also induce increased hair growth: major thoracic surgery can be followed by a unilateral hypertrichosis ("hemitrichosis"), which can be reproduced in dogs and does not seem to result from an increased blood flow (e.g., due to the severance of sympathetic nerve fibers) (Kobayshi *et al*, 1958). Also, hyperplasia of the remaining dorsal root ganglia following partial neurectomy in opossum pups leads to skin hyperinnervation, an increase in epidermal thickness, and a precocious development of HFs (Jones and Munger, 1987). Experimentally induced sympathetic hyper-innervation of blood vessels in rabbit skin may also be accompanied by localized, excessive hair growth (cf. Crowe *et al*, 1993).

Finally, there is a relative hyperinnervation of the distal HF, whose neural network exhibits a corona of longitudinally and circularly oriented nerve fibers located at the level of the follicle isthmus and bulge (Winkelmann, 1960; Halata, 1993) (cf. **Figs 1, 2**). Traditionally, this has been explained with the functions of the HF as a sensory, tactile organ: the distal follicle experiences the largest degree of hair shaft displacement by external objects such as insects or fingertips so that hair shaft displacement is most effectively recorded at this follicular level. The bulge region, however, contains epithelial stem cells (Cotsarelis *et al*, 1990). Given the prominence of peptidergic nerve fibers around this follicle region (Hartschuh *et al*, 1983; Bjorklund *et al*, 1986; Karanth *et al*, 1991; Katoh *et al*, 1991; Ebara *et al*, 1992; Karanth, 1994) and in view of the recognized growth-modulatory properties of many NPs and NTs (see this supplement; Rozengurt, 1991, Paus *et al*, 1994c, 1995; Ansel *et al*, 1996; Crawley and McLean, 1996; Baraniuk, 1997), it is reasonable to ask whether the peculiar fiber arrangement around the distal outer root sheath, at least in part, serves to modulate epithelial stem cell functions by the controlled release of NPs and NTs.

Table II. Neural Mechanisms of Hair Growth Control? Arguments Pro and Contra

Contra
Development of follicle rudiments in raft cultures of embryonal skin
Hair growth even after follicle transplantation and in organ culture
Pro
Abnormal hair growh (e.g., follicle atrophy) after neuralectomy, limb paralysis, nerve degeneration
"Hemitrichosis" after thoracic surgery
Precocious follicle development after neuralectomy
"Stress"-induced alopecia areata
"Overnight" graying
Unusual density and arrangement of follicle innervation in stem cell region

For references, see Kobayashi *et al*, 1958; Winkelmann, 1960; Giacometti and Montagna, 1967; Whitlock, 1976; Jones and Munger, 1987; Hashimoto *et al*, 1990; Holbrook and Minami, 1991; Li *et al*, 1992b, 1992c; Halata, 1993; Lichti *et al*, 1993; Philpott *et al*, 1994.

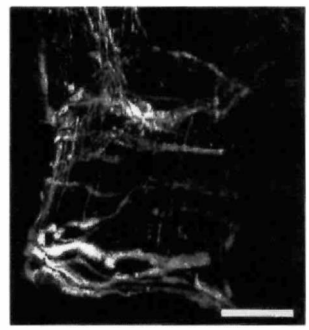

Figure 2. Circular and longitudinal perifollicular nerve fibers (follicular network B -FNB). PGP 9.5-immunoreactive longitudinal and circular fibers of the FNB of an anagen VI pelage follicle in the back skin of C57BL/6 mouse skin are shown (confocal microscopy). (cf. Botchkarev *et al*, 1997a) *Scale bar*, 20 μm.

THE MURINE HAIR CYCLE AS A MODEL: LEADS AND LESSONS FROM C57BL/6 MICE

In contrast to the mosaic cyling of human HFs, follicle cycling in the first months of murine postnatal life is well-synchronized. Also, follicle morphogenesis can still be studied in neonatal mouse skin because most pelage HFs develop in the peri- and neonatal period (Hardy, 1992; Vielkind *et al*, 1995). Therefore, spontaneous changes in follicle innervation can be correlated here with defined stages in the epithelial morphogenesis and remodeling of the HF. Furthermore, an ever-increasing number of mouse mutants with functional deletion or overexpression of a gene coding for NTs, NP, or their receptors (Bothwell, 1995; Lewin and Barde, 1996), or for proteins relevant to nerve fiber structure, function, and sprouting (cf. Smith, 1996; see this supplement) provides incisive research tools for dissecting the relative significance of defined neurobiologic parameters in piloneural interactions. In particular, the C57BL/6 mouse model for hair research (Paus *et al*, 1994a, 1994b, 1994e) has recently provided interesting new leads to the characteristics of piloneural interactions.

The Architecture of Hair Follicle Innervation Shows Hair Cycle-Dependent Plasticity One basic, phenomenologic approach to enter into a dissection of piloneural interactions is to carefully check whether the cyclic transformations of the HF correlate with any structural changes in HF innervation. It is not unreasonable to expect that the dramatic epithelial tissue remodeling seen during the hair cycle (Paus, 1996) is associated with a corresponding remodeling of tissue innervation. On the basis of histochemical studies, however, it is widely thought that the perifollicular neural plexus of mature HFs does not undergo any remodeling during the hair cycle. Instead, the follicle innervation network is believed to simply collapse during the catagen-telogen transformation and to get re-extended into its original arrangement by the new, growing anagen hair bulb (Winkelmann, 1960; Giacometti and Montagna, 1967; Winkelmann, 1988).

Studying the depilation-induced murine hair cycle and employing sensitive immunohistologic techniques for nerve fiber demarcation (PGP 9.5, neurofilament 150 expression), we could recently show that this dogma, at least in mice, is misleading (Botchkarev *et al*, 1997a). The architecture of HF innervation in adolescent mice shows striking hair cycle-dependent plasticity, mainly in one selected region [i.e., the circular nerve fibers around the HF isthmus, the so-called "follicular network B " (FNB)]. Substantial innervation changes occur even in interfollicular murine skin during the hair cycle: the circular, but not the longitudinal, fibers in "follicular network B" as well as irregularly arranged fibers in "follicular

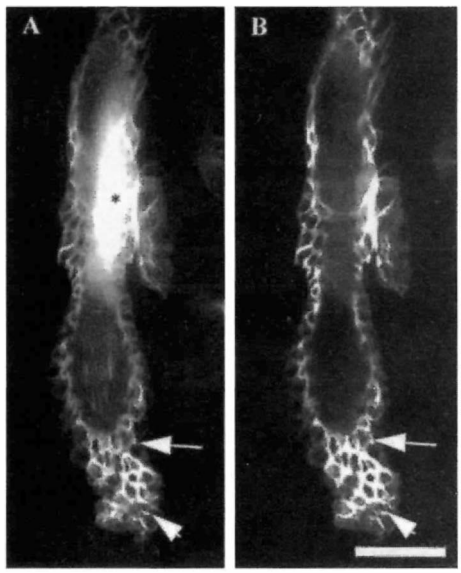

Figure 3. The HF as a source and target of neurotrophins. *(A)* NGF+ cells in a murine telogen HF. (immunofluorescence; * indicates autofluorescence of hair shaft; -▷ indicates dermal papilla; —▷ indicates distal border of hair germ cells). *(B)* TrkA+ cells in the same HF section. *Scale bar*, 25 μm.

network A" significantly increase in number during the earliest stages of anagen development (anagen II) **(Figs 1, 2)**.

At the same time, the epidermis and interfollicular dermis of early anagen skin become "hyper-innervated," and the circular nerve fibers of FNB upregulate their expression of growth-associated protein-43 (GAP-43) and neural cell adhesion molecules as an indicator of active fiber remodeling and sprouting. The innervation density of FNB declines again during the later stages of anagen, whereas the three horizontally arranged nerve plexus of murine skin, which get increasingly separated from each other by the growing anagen hair bulb, appear to retain a fairly constant architecture throughout the cycle. A large epithelial follicle compartment, the anagen hair bulb, however, which does not even exist during telogen, may receive new nerve fibers in 16% of anagen VI follicles **(Fig 1)** (Botchkarev *et al*, 1997a). How may these nerve rearrangements be initiated and orchestrated, and are they functionally significant for the control of HF cycling?

The Hair Follicle Is a Source and Target of Neurotrophins As a first step toward answering these questions, we have studied the skin expression of various NTs (NGF, NT-3, NT-4, BDNF) and their receptors (tyrosine kinase (Trk) A, TrkB, TrkC, p75NTR) during HF cycling and have just generated an immunohistologic profile of NT and NT receptor expression during murine HF development and cycling.[1,2] This reveals that defined HF compartments are not only a prominent source of NTs **(Fig 3*A*)**, but that they also are likely NT targets because they show a distinct pattern of NT receptor expression **(Figs 3*B*, 5)**. Correspondingly, we detected significant hair cycle-dependent changes in NGF gene and protein expression, studying NGF mRNA steady-state levels (reverse transcriptase-polymerase chain reaction) and NGF protein content (enzyme-linked immunosorbent assay, western blot) in full-thickness mouse skin homogenates.[2]

This does not come as a surprise because mouse and human keratinocytes are known to express NGF and NT receptors (Bothwell, 1991; Vega *et al*, 1994; Akiyama *et al*, 1996; Bronzetti *et al*, 1996; Shibayama and Koizumi, 1996), and because there is selective NT receptor expression on epithelial cells in related developmental systems, such as during tooth morphogenesis (Luukko *et al*, 1996). Nonetheless, it highlights the importance of systematic exploration of the role of NTs in the control of epithelial cell proliferation, differentiation, and apoptosis (cf. Paus *et al*, 1994d, Zhai *et al*, 1996; Pincelli and Yaar, 1997).

Intraepidermal and intrafollicular overexpression of NGF or NT-3 under the K14 promoter induces substantial skin hyperinnervation and epidermal hyperplasia in transgenic mice (Albers *et al*, 1994, 1996; cf. Davis *et al*, 1993, 1994). Interestingly, in organ-cultured murine HFs growing in their intact skin environment, keratinocyte proliferation in resting (telogen) follicles was stimulated by NGF, whereas that of maximally proliferating (i.e., anagen II) follicles was inhibited (Paus *et al*, 1994d). These opposite effects of NGF suggest that there are cell cycle- and/or differentiation-related changes in NGF receptor expression by murine keratinocytes. Reportedly, p75NTR is the first growth factor receptor expressed by those human embryonal skin fibroblasts that condense to form the later dermal papilla of the follicle (Holbrook and Minami, 1991). In mature murine anagen VI follicles, we have just detected TrkB and TrkC expression by dermal papilla cells[1,3,4] (cf. **Fig 5**).

It is conceivable, therefore, that the HF itself directs the changes in follicular innervation that it may require for optimal follicle cycling and growth as well as for executing its tactile functions by generating and releasing selected NT. In addition, NT may modulate functions of the follicle epithelium indirectly by altering, for example, the secretion of epithelial morphogens and growth factors by dermal papilla fibroblasts. Such NTs targeting the dermal papilla could arise not only from Schwann cells and nerves, but from the follicle epithelium itself (cf. **Fig. 5**).

Most recently we have collected evidence suggesting that NT-3 and BDNF are involved in the regulation of murine HF development and cycling.[3,4] HF morphogenesis is accelerated in the skin of NT-3 overexpressing mice and retarded in NT-3 heterozygous knockout mice, respectively. Moreover, precocious catagen development and shortening of anagen occurs in NT-3 overexpressing mice, and the back skin follicles of BDNF knockout mice are still in their first catagen phase when those of wild-type mice have already entered the first telogen stage.[3,4]

Peptidergic Signaling in Murine Skin Changes during the Hair Cycle Not only the physical structure of defined sectors of murine HF innervation changes during the hair cycle (Botchkarev *et al*, 1997a), but also their expression of NPs and NTMs.

For example, the substance P (SP) content of murine skin fluctuates significantly during the induced hair cycle, with maximal SP skin levels occurring in early anagen, and minimal ones in catagen skin (Paus *et al*, 1994c). Most, if not all, intracutaneous SP is thought to be synthesized in dorsal root ganglia and then transported into sensory nerve fiber terminals (Maggi, 1995; Smith, 1996). Therefore, these hair cycle-dependent fluctuations in skin SP may reflect some form of spinal-follicular communication that affects SP gene expression and/or SP synthesis in dorsal root neurons, and/or the transport of SP into the skin (Paus *et al*, 1994c). Alternatively, hair cycle-dependent fluctuations in the skin activity of SP-degrading enzymes may explain this phenomenon. This is unlikely, however, because the activity of the key tachykinin-degrading enzyme, neutral endopeptidase (NEP), does not change

[1] Botchkarev VA, Peters EMJ, Eichmüller S, Botchkareva NV, Paus R: The hair follicle as a source and target of neurotrophic factors. *J Invest Dermatol* 107:507, 1996 (abstr).

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[4] Botchkarev VA, Lewin GR, Albers KM, Botchkareva NV, Peters EMJ, Paus R. Neurotrophins and murine hair follicle morphogenesis: expression patterns of NT-3, NT-4, BDNF, TrkB and TrkC and indications for a functional role in hair follicle development and regression. *J Invest Dermatol* 108:620, 1997 (abstr).

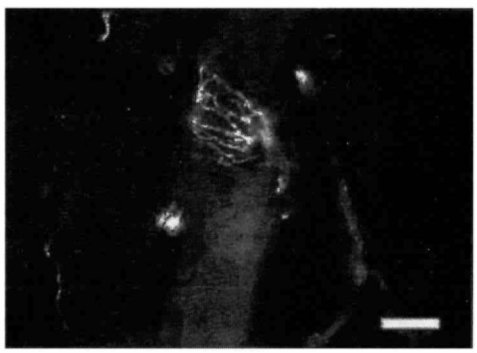

Figure 4. Prominent calcitonin gene-related peptide (CGRP)+ nerve fibers in FNB. The figure shows CGRP-immunoreactive, peptidergic, sensory nerve fibers around the level of the HF isthmus and bulge region of a C57BL/6 pelage hair follicle, where epithelial stem cells are located (Cotsarelis *et al*, 1990). Note that in C57BL/6 mice, practically no substance P+ fibers can be detected in the vicinity of the HF (in contrast to the abundance of SP+ fibers in the FNB region of the human perifollicular neural network). *Scale bar*, 25 μm.

in a manner that would explain the above fluctuations in SP skin levels. Yet, even the activity of this membrane-bound NP-degradation system, which controls the functional effects of tachykinins (cf. Grady *et al*, 1997), is regulated in a hair cycle-dependent manner: maximal NEP activity occurs in mid-anagen, and minimal activity occurs in early and late anagen skin (Paus *et al*, 1994c).

Recently, we noticed that the number of SP-immunoreactive nerve fibers in mouse skin also changes in a hair cycle-dependent manner[5] (Botchkarev *et al* 1997b). A maximum of SP+ fibers was observed in early anagen skin, and a minimum in telogen. In striking contrast to human perifollicular peptidergic nerves, however, many of which are prominently SP+ (Bjorklund *et al*, 1986; Hordinsky *et al*, 1995; Hordinsky and Ericson, 1996), extremely few SP+ nerve fibers were noted in close proximity to HFs in C57BL/6 mouse skin. Instead, most perifollicular peptidergic fibers were calcitonin gene-related peptide (CGRP)+ **(Fig 4)**; a maximum of CGRP+ fibers was observed in early anagen skin, and a minimum in telogen and catagen skin (Botchkarev *et al*, 1997b). These results question whether SP is a major piloneural communication signal in mice and raise the possibility that other NP detected in the pilosebaceous unit [e.g., ACTH (Slominski *et al*, 1993), β-endorphin (Furkert *et al*, 1997)] may functionally be more important under physiologic conditions.

Some Neuropeptides and Neurotransmitters Modulate Hair Follicle Cycling *in Vivo* Selected skin NPs alter murine HF cycling *in vivo*. Subcutaneously implanted pellets releasing SP induce anagen (Paus *et al*, 1994c), and even subnanomolar concentrations of SP significantly stimulate HF keratinocyte proliferation in murine skin organ culture (Paus *et al*, 1995). Most recently, we have found that intracutaneous SP or ACTH injections also induce localized HF regression (catagen).[6]

Intracutaneous ACTH injections into telogen back skin can induce anagen in mice (Paus *et al*, 1994e), as do intracutaneous injections of neurotoxic agents that deplete endogenous NP or NTMs stores in the skin (capsaicin, 6-hydroxy-dopamine, guanethidine)[7] (Paus *et al*, 1994c). Capsaicin injection also induces premature catagen development in mice.[6] Interestingly, capsaicin- or SP-induced catagen HF show signs of follicle dystrophy, accompanied by some degree of alopecia localized to the injection site.[6] Thus, massive exposure toward selected NPs (either by injecting them or by depleting endogenous NP stores) can be associated with follicle damage and hair loss. Could this be one of the pathomechanisms underlying the rare cases of alopecia that have credibly followed extreme psychoemotional stress?

Autonomic-Nervous Controls of Hair Growth May Exist In order to address the role of autonomic-nervous signaling in hair growth control, we have studied tyrosine hydroxylase antigen as a marker for adrenergic nerves and choline acetyl transferase as an immunohistologic marker for cholinergic nerves and have visualized the noradrenaline content of nerve fibers by paraformaldehyde or glyoxylic acid condensation. Once again, substantial hair cycle–associated changes were noted: for example, the number of tyrosine hydroxylase-positive and noradrenaline-containing nerve fibers as well as that of choline acetyl transferase-positive fibers significantly increases in anagen compared to telogen skin; noradrenaline-positive and tyrosine hydroxylase-positive nerve fibers then decline again toward catagen.[7] That the noradrenaline-depleting agents 6-hydroxy-dopamine and guanethidine induce anagen[7] supports the concept that autonomic-nervous signals can modulate hair growth, in principle (compare also Asada-Kubota, 1995). This is supported by the clinical observation that β-blockers and amphetamines, for example, can cause a telogen effluvium.

Mast Cell-Nerve Interactions May Be Involved in the Control of HF Cycling and/or Innervation Substantial evidence now suggests that mast cells (MCs) have hair growth-modulatory properties (Botchkarev *et al*, 1995; Maurer *et al*, 1995), namely that they are important for both anagen (Paus *et al*, 1994e) and catagen development in mice.[8] Skin MCs frequently are found in close proximity to cutaneous nerve fibers (e.g., Naukkarinen *et al*, 1993), possibly in order to facilitate bidirectional MC-nerve interactions. For example, skin NPs like SP induce cytokine release (Ansel *et al*, 1993) and MC degranulation in mice (Paus *et al*, 1995), whereas MCs can secrete NGF, induce the axon reflex, and produce NP-degrading proteases (Kiernan *et al*, 1972; Caughey *et al*, 1988; Foreman, 1988; Leon *et al* 1994). Therefore, it is interesting to note that MC-nerve contacts change during the murine hair cycle and appear to be nonrandom.

In telogen and early anagen skin, MCs preferentially contact CGRP+ or SP- and CGRP-double+ sensory nerve fibers; and during late anagen, there is a significant increase in the number of close contacts between MCs and andrenergic (tyrosine hydroxylase-positive) fibers (compared to telogen values), whereas contacts between MC and peptide histidine-methionine-positive or choline acetyl transferase-positive nerve fibers peak during catagen (Botchkarev *et al*, 1997b). In view of the hair growth-modulatory properties of MC, on the one hand (Maurer *et al*, 1995), and the indications supporting neural mechanisms of hair growth control, on the other **(Table II)**, it is tempting to speculate that MC-nerve interactions are functionally relevant to the control of HF cycling and/or innervation.

PILONEURAL INTERACTIONS: PRINCIPLES AND PERSPECTIVES

Several levels of communication between cutaneous nerve endings and their target cells in the HF can be envisioned **(Fig 5)**.

Most importantly, direct "trophic" effects of skin nerves on the

[5] Eichmüller S, Botchkarev VA, Johansson O, Paus R. Hair cycle-dependent rearrangement of murine skin innervation. *J Invest Dermatol* 106:889, 1996 (abstr).

[6] Maurer M, Peters EMJ, Fischer E, Botchkarev VA, Eichmüller S, Paus R. The role of neuropeptides in murine hair cycle modulation: induction of hair follicle regression by capsaicin and substance P. *J Invest Dermatol* 107:489, 1996 (abstr).

[7] Peters EMJ, Maurer M, Botchkarev VA, Eichmüller S, Paus R. Autonomic innervation of murine skin: hair cycle-dependent remodelling and hair growth induction by drugs modulating adrenergic function. *J Invest Dermatol* 107:488, 1996 (abstr).

[8] Maurer M, Eichmüller S, Botchkarev VA, Peters EMJ, Paus R: Modulation of murine hair follicle regression by neonatal capsaicin treatment. *J Invest Dermatol* 106:889, 1996 (abstr).

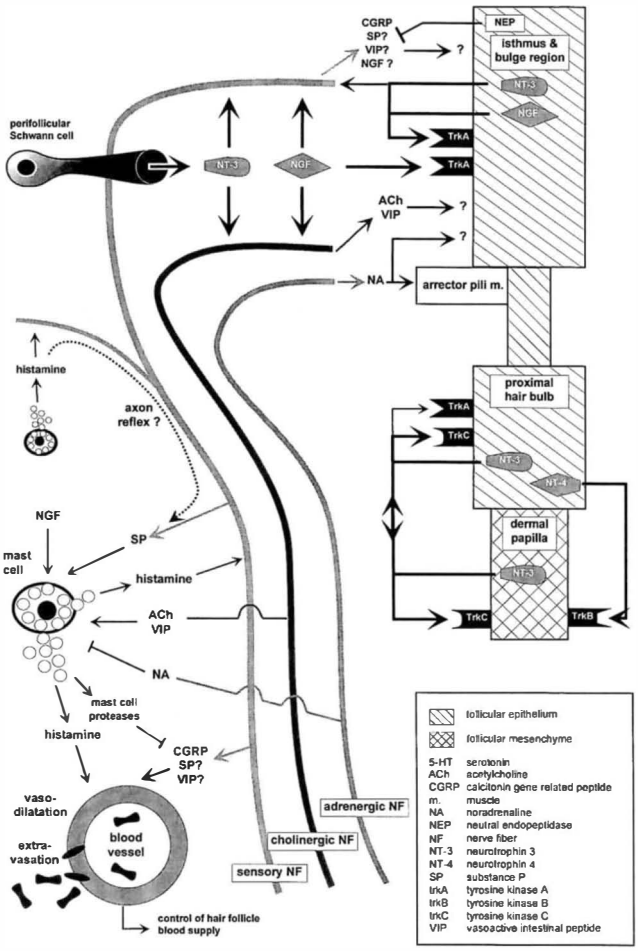

Figure 5. Hypothetical, direct and indirect piloneural interactions in murine skin. See text for explanation.

HF need to be distinguished from indirect ones (**Table I**), such as changes in the local blood supply caused by nerve damage or by hyper-innervation. *In vivo*, it is quite difficult, however, to differentiate between follicle atrophy due to inadequate skin perfusion as a consequence of disturbed vasomotoric control by damaged peripheral nerves and a lack of direct, trophic growth stimuli secreted by these nerves. NPs, such as CGRP and SP, released from afferent nerves in the skin are potent vasodilatory agents and/or induce plasma extravasation and leukocyte migration into the skin (Maggi *et al*, 1987; Matis *et al*, 1990; Xu *et al*, 1992; Maggi, 1995; Morris, 1995; Crawley and McLean, 1996; Baraniuk, 1997). Also, adrenergic transmitters, such as noradrenaline released from sympathetic nerve fibers in the skin, cause strong vasoconstriction (White and Udwadia, 1975; Raff and Neumann, 1985; Burnstock and Ralevic, 1994; Smith, 1996), and sensory and autonomic nerve fibers in the skin can differentially release a multitude of vasoactive peptides (Leeman *et al*, 1991; Lotti *et al*, 1995; Morris, 1995; Ansel *et al*, 1997; Wallengren, 1997).

Thus, the most profound "trophic" effects of cutaneous nerve fibers on HF growth may well be exerted via regulating the supply of nutrients and oxygen to the HF — an organ that, due to its very high metabolic demands and proliferative activity during anagen, is probably very sensitive to perfusion changes. Yet, a host of other indirect effects of neural signals on HF keratinocyte proliferation, differentiation, and apoptosis must be considered (**Table I, Fig 5**). NP, NTM, and NT alter multiple functions of hematopoietic cells relevant to HF biology such as MCs, macrophages, Langerhans cells, and T cells (cf. Leeman *et al*, 1991; Crawley and McLean, 1996; Baraniuk, 1997; see Ansel *et al*, 1997; Torii *et al*, 1997). In particular, the modulation of MC and macrophage activities by NPs such as SP and CGRP deserves special attention because both cell populations have been implicated in hair growth control (Maurer *et al*, 1995; Paus, 1996, 1997).

Furthermore, NPs, NTMs, and NTs released from peri- or intrafollicular nerve fibers or their glia might alter fibroblast functions in the dermal papilla, the mesenchymal "control center" of the HF (Hardy, 1992; Paus, 1996) [note that the first evidence that NPs have growth factor properties arose from studies on fibroblast populations (cf. Nilsson *et al*, 1985; Rozengurt, 1991)]. With the exception of vibrissae follicles, however, the proximal HF in mice is only weakly innervated, if at all (Botchkarev *et al*, 1997a) (cf. **Fig. 1**). Therefore, at least in murine pelage follicles, indirect NP and NT effects on hair bulb keratinocytes (i.e., release from perifollicular nerves, and modulation of dermal papilla fibroblast functions) are probably not a major factor in piloneural interactions (**Fig 5**).

Rather, NPs, NTM, and NTs may directly stimulate appropriate receptors on HF keratinocytes, because keratinocytes, which express high-affinity receptors for all these classes of neural signaling molecules (Grando *et al*, 1993; Schallreuter *et al* 1993, 1995; Grando, 1997; Pincelli and Yaar, 1997; Ansel *et al*, 1997). Here, one needs to consider that skin nerve fibers may not only secrete NP and NTM, but may also release NTs such as NGF and NT-3 previously taken up via NT receptors (Lewin and Barde, 1996; Bothwell, 1997). In addition, the Schwann cells, ensheathing perifollicular nerve fibers, are a rich source of secreted growth factors, including NTs (Lewin and Barde, 1996; Smith, 1996). Finally, enzymes that rapidly degrade secreted NPs such as neutral endopeptidase may be secreted by or expressed on the cell surface of HF keratinocytes, thereby controlling the level of follicle keratinocyte stimulation by NPs secreted by perifollicular nerve fibers.

It remains to be seen whether neural mechanisms of hair growth control exist. The bulk of the currently available evidence are affirmative. How relevant are they clinically? This is still too early to say and definitely will be much more difficult to probe in patients than in mice. At least, specific signaling pathways of piloneural communication can now be defined (**Fig 5**). Specifically, it appears promising to screen human scalp skin specimens from patients with telogen effluvium or alopecia areata for abnormalities in the parameters of piloneural signaling listed in **Fig 5**, compared to normal controls. Initial reports on lesional alopecia areata follicles (Hordinsky *et al*, 1995; Hordinsky and Ericson, 1996), which have uncovered abnormalities in the peptidergic innervation of these follicles, invite one to extend such analyses to other forms of hair loss reputed to be associated with psychoemotional stressors or neurologic abnormalities.

If skin nerves really exert "trophic" roles, one would expect a certain level of continuous NP-, NT- and/or NTM-secretion in order to sustain epithelial homeostasis. Yet, this putative baseline secretion *in vivo*, not to mention the underlying enzymatic, transcriptional, and secretory controls, is virtually unknown. These need to be characterized in normal skin as well as in inflammatory, hyper-proliferative, and atrophic skin diseases, where baseline secretion may be abnormal. The baseline NP, NT, and NTM levels, and the skin expression of corresponding receptors, certainly are interesting targets for pharmacologic manipulation.

In addition, locally administered NPs, namely those with a very short half-life and a correspondingly low risk of systemic side effects, are attractive candidate "hair drugs," the more so if effective doses can be delivered by "follicle-targeted" liposome preparations (e.g., Li *et al*, 1992a; Lieb *et al*, 1992; Lauer *et al*, 1996). Specifically, our data from the C57BL/6 mouse model encourage one to explore selected NP as anagen-inducing agents, which may be of use for treating telogen effluvium and androgenetic alopecia. In contrast, very high doses of some NPs may be employed to induce the shedding of unwanted hair, e.g., in hypertrichosis or hirsutism (cf. Paus, 1996).

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