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DIFFERENTIAL EXPRESSION OF HPA AXIS HOMOLOG IN THE SKIN

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Summary

Human skin expresses elements of the hypothalamo-pituitary-adrenal (HPA) axis including pro-opiomelanocortin (POMC), corticotropin releasing hormone (CRH), the CRH receptor-1 (CRH-R1), key enzymes of corticosteroid synthesis and synthesizes glucocorticoids. Expression of these elements is organized in functional, cell type-specific regulatory loops, which imitate the signaling structural hierarchy of the HPA axis. In melanocytes and fibroblasts CRH-induced CRH-R1 stimulation upregulates POMC expression and production of ACTH through activation of cAMP dependent pathway(s). Melanocytes respond with enhanced production of cortisol and corticosterone, which is dependent on POMC activity. Fibroblasts respond to CRH and ACTH with enhanced production of corticosterone, but not cortisol, which is produced constitutively. Organ-cultured human scalp hair follicles also show a fully functional HPA axis equivalent, including cortisol synthesis and secretion and negative feedback regulation by cortisol on CRH expression. Thus differential, CRH-driven responses of skin reproduce key features of the central HPA axis at the tissue/single cell levels.

Keywords

CRH; CRH-R1; POMC; cutaneous steroidogenesis; cutaneous P450scc; stress response

1. Introduction

1.1.HPA axis

Conventional wisdom sees the vertebrate CNS as controlling the endocrine system, including a battery of systemic responses to psychoemotional stress: the response to both acute, and chronic sustained stress is mediated humorally, through involvement of the hypothalamic pituitary adrenal (HPA) axis (Chrousos and Gold, 1992). This pathway, when activated by

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stress-sensing central circuits, proceeds with hypothalamic production and release of CRH which stimulates pituitary CRH-R1 (Hillhouse and Grammatopoulos, 2006; Perrin and Vale, 1999). A CRH-R1 led signal transduction pathway enhances the production and secretion of the anterior pituitary-derived POMC peptides, ACTH and β -endorphin (Hillhouse and Grammatopoulos, 2006; Smith and Funder, 1988). Upon ACTH release into the systemic circulation, it activates the MC2 receptors (MC2-R) of the adrenal gland inducing production and secretion of corticosterone (rodents) or cortisol (humans). These steroids counteract the actions of stressors, mobilize adequate responses and energy reserves, and buffer tissue damage with powerful antiinflammatory activity. The same glucocorticoids act to tone-down the stress response by interacting directly with receptors in the CNS or, with receptors in the anterior pituitary to attenuate CRH and POMC peptide production. Thus, the HPA axis encodes an adaptive response devoted to the stabilization and restoration of general homeostasis.

1.2. The skin as a neuroendocrine organ

For more than a decade, however, a body of evidence has been accumulating that endocrine stress responses are not necessarily under CNS control, and also do occur at the peripheral tissue level, outside of the classical HPA axis. This has become most evident, and has been best explored, in mammalian skin.

The strategic location of the skin as a barrier between the environment and the internal milieu determines its critical function in the preservation of body homeostasis (Slominski, 2005; Slominski and Wortsman, 2000; Slominski et al., 2000d). The skin is continuously exposed to many hostile environmental factors (chemical and biological) and to acute transfers of solar, thermal or mechanical energy (Slominski and Pawelek, 1998; Slominski and Wortsman, 2000; Slominski et al., 2000d). In part as a mechanism of defense, skin cells produce hormones, neurotransmitters and neuropeptides and express cognate functional receptors (Slominski, 2006; Slominski et al., 2004b; Slominski and Wortsman, 2000; Slominski et al., 2000d). Besides being produced locally in epidermal, adnexal and dermal cells, hormones and neurotransmitters are also released *in situ* from cutaneous nerve endings (Slominski and Wortsman, 2000). These profound neuroendocrine activities of skin complement its exocrine activities, performed by the adnexal structures e.g., eccrine, apocrine and sebaceous glands and hair follicles (Slominski and Wortsman, 2000; Stenn and Paus, 2001; Zouboulis and Bohm, 2004). Exocrine activities function to strengthen the epidermal barrier and control thermoregulation, or participate in the defense against microorganisms, or in social communication (Slominski, 2005; Slominski et al., 2004b; Slominski and Wortsman, 2000; Stenn and Paus, 2001; Zouboulis and Bohm, 2004).

Upon disruption, there are several mechanisms for the prompt restoration of the skin structural and functional integrity. Such mechanisms are represented by the barrier forming properties of the epidermis, the secretory and protective activities of adnexal structures, skin immune and pigmentary systems as well as by vascular and mesenchymal components of the dermis (Bohm and Zouboulis, 2004; Luger et al., 1999; Slominski, 2005; Slominski et al., 2004b; Slominski and Wortsman, 2000; Stenn and Paus, 2001; Zouboulis and Bohm, 2004). It is these mechanisms, as well as the precise coordination of their responses, that appear to be functionally served by local cutaneous neuroendocrine activities (Slominski, 2005; Slominski and Wortsman, 2000; Slominski et al., 2000d). Thus, cutaneous signals relayed to this local neuroendocrine system may trigger cascades of responses directed at maintaining local, and hence global, homeostasis (Slominski et al., 2004a; Slominski et al., 2004b; Slominski and Wortsman, 2000; Slominski et al., 2001; Slominski et al., 1999c; Zouboulis and Bohm, 2004).

2. Cutaneous elements of the hypothalamic-pituitary axis

2.1. Cutaneous CRH signaling system

Though, historically, proopiomelanocortin-derived peptides like α -MSH, ACTH and β -endorphin were the first stress-related mediators detected in mammalian skin (Slominski et al., 1993), the concept of the intracutaneous establishment of a peripheral HPA axis equivalent became intuitive by the discovery of CRH in skin and several of its constituent cell populations (Slominski, 2006; Slominski and Mihm, 1996).

CRH and related peptides—Skin cells produce CRH and the related peptides urocortin (urc) 1 and 2 (Roloff et al., 1998; Slominski, 2006; Slominski et al., 1995; Slominski et al., 1996c; Slominski et al., 1998b; Slominski et al., 2000c; Slominski et al., 1999c). In human skin, the production of CRH is associated with changes in the expression of the CRH gene, which is stimulated by UV radiation and forskolin and inhibited by dexamethasone (Slominski et al., 1996a; Slominski et al., 1998b; Zbytek et al., 2006). In mouse skin, there is hair-cycle-dependent production of CRH, even though corresponding changes in gene expression could not be detected (Roloff et al., 1998; Slominski et al., 1996c; Slominski et al., 2001; Slominski et al., 1999c). However, mouse skin expresses the gene for urocortin and produces the respective peptide (Slominski et al., 2000b). Cutaneous expression of CRH and urc 1 and 2 is both cellular compartment- and species-specific (Slominski et al., 2004a).

CRH receptors—CRH-R1 and CRH-R2 are expressed in human (Slominski, 2006; Slominski et al., 1995; Slominski et al., 2000d; Slominski et al., 2001; Slominski et al., 1999c) and rodent (Pisarchik and Slominski, 2002; Roloff et al., 1998; Slominski et al., 1996c; Slominski et al., 2000d; Slominski et al., 1999c) skin, with species-specific compartmental localization. In the human skin, CRH-R1 is predominantly expressed in the epidermis with CRH-R1 α being the major isoform present (Slominski et al., 2004a). The cutaneous CRH-R1 is activated by CRH or urocortin and its intracellular signal transduction pathways are coupled to cAMP, IP3 or Ca (Fazal et al., 1998; Slominski et al., 2006b; Slominski et al., 1999c; Slominski et al., 2000e; Wiesner et al., 2003; Zbytek and Slominski, 2005). UV radiation and factors raising intracellular cAMP increase CRH-R1 α expression and change the pattern of isoform expression (Pisarchik and Slominski, 2001; Slominski et al., 2001). CRH-R1 participates in the regulation of skin cell proliferation, apoptosis, differentiation and immune activities (Carlson et al., 2001; Quevedo et al., 2001; Slominski, 2005; Slominski et al., 2000d; Slominski et al., 2006b; Zbytek et al., 2004; Zbytek and Slominski, 2005).

2.2. Cutaneous POMC system

POMC system—It is well documented that the skin can transcribe the POMC gene, process it (alternative splicing) and translate the final message into the POMC precursor protein in a regulated fashion that is species dependent (Slominski et al., 1993; Slominski et al., 2000d). The skin also contains the full machinery for POMC processing (PC1, PC2 convertases and 7B2 protein) with generation of ACTH or α -MSH, and β -LPH or β -endorphin as final products, depending on the cellular compartment and cell type (Bohm et al., 2005; Luger et al., 1999; Paus et al., 1999; Slominski et al., 1999b; Slominski et al., 1993; Slominski et al., 2004b; Slominski and Wortsman, 2000; Slominski et al., 2000d; Wintzen and Gilchrist, 1996). In human skin, the process can be upregulated by UVR and factors increasing cAMP levels, while it is inhibited by several growth factors (Luger et al., 1999; Slominski and Pawelek, 1998; Slominski et al., 2004b; Slominski et al., 2000d). In mouse skin, POMC expression and the production of functional POMC-derived peptides is inhibited by glucocorticoids, and coupled to hair follicle cycling (Ermak and Slominski, 1997; Paus et al., 1999; Slominski et al., 1998a; Slominski et al., 2000d; Slominski et al., 2005d).

Receptors for POMC peptides—Several key skin cell populations express the functional, cognate receptors activated by the above neuropeptides (Bohm and Zouboulis, 2004; Luger et al., 1999; Slominski et al., 2004b; Slominski and Wortsman, 2000; Slominski et al., 2000d; Zouboulis et al., 2002). Specifically, MCR1, 2 and 5, as well as receptors for β -endorphin have been identified in human and rodent skin cells (Slominski et al., 2004b; Slominski et al., 2000d). In cell type- and skin compartment-specific manners, activation of the above receptors regulates or modifies, for example, epidermal barrier functions, hair growth and pigmentation as well as hair follicle immunology, melanin pigmentation, secretory activity of adnexal structures, local (and perhaps systemic) immune activity, as well as activity of the dermal vascular and fibroblast systems. Of note, UVB-induced melanogenesis is, at least in part, mediated by upregulation of the MSH receptor system, which in turn can be activated by both melanocortins and ACTH peptides (Chakraborty et al., 1999; Pawelek et al., 1992; Slominski and Pawelek, 1998). In rodents, expression of MCR1 is hair-cycle-dependent with the highest expression seen during anagen and the lowest during the telogen, while expression of MCR2 remains constant throughout the hair cycle (Ermak and Slominski, 1997).

3. Cutaneous steroidogenesis

3.1. Cytochrome P450scc system

Cytochrome P450scc, a mitochondrial enzyme that is the product of the CYP11A1 locus, was considered until recently to use cholesterol as sole substrate in hydroxylating and side chain cleaving reactions to produce pregnenolone, the substrate for steroid hormones. It is now clear that cytochrome P450scc can also efficiently use other substrates that include 7-DHC, ergosterol, and vitamins D2 and D3, to produce steroidal 5, 7-dienes or hydroxyderivatives of vitamin D or ergosterol (Guryev et al., 2003; Slominski et al., 2006a; Slominski et al., 2005b; Slominski et al., 2005c; Slominski et al., 2004c). This is pertinent since mammalian skin expresses the CYP11A1 gene and the actual P450scc protein (Slominski et al., 2004c). This expression was detected in a wide assortment of mouse and human skin samples, subcutaneous adipose tissue, and in normal, immortalized and malignant epidermal and dermal cell lines. Moreover, an alternatively spliced CYP11A1 was detected in keratinocytes, squamous cell carcinoma and melanoma cells (GeneBank # AY603498)(Slominski et al., 2004c). The genes for components of the P450scc system, adrenodoxin (FDX1) and adrenodoxin reductase (FDXR), are also expressed in human and mouse skin and cultured skin cells, with the resulting proteins being recognized by specific adrenodoxin and adrenodoxin reductase antibodies, respectively (Slominski et al., 2004c).

The transport of cholesterol substrate for P450scc into mitochondria is performed by specific cholesterol transporting proteins, StAR or MLN64 (Bose et al., 2000; Stocco, 2000; Watari et al., 1997). Using specific antibodies that recognize a common epitope for both MLN64 and StAR (Bose et al., 2000), we detected the expected protein with MW of 55 kDa (Slominski et al., 2004c) corresponding to MLN64 (Uribe et al., 2003) in human and rodent skin samples. Additional immunoreactive proteins of lower MW (37 kDa and 18 kDa) could represent products of MLN64 processing and/or the full-length StAR protein (Bose et al., 2000; Stocco, 2000; Watari et al., 1997). This suggests that mammalian skin cells are biochemically equipped for cholesterol transport as the basis for steroidogenesis. This was confirmed when isolated mitochondria from skin cells were shown to be able to transform cholesterol into pregnenolone and progesterone, albeit at low efficiency (1% of placenta) (Slominski et al., 2004c). Cultured skin cells (squamous cell carcinoma and melanoma) transformed 22R-hydroxycholesterol into pregnenolone. Pregnenolone was only observed in the presence of 8 μ M cyanoketone (a 3 β HSD inhibitor); in its absence pregnenolone was rapidly metabolized, most likely via the delta-4 pathway (Slominski et al., 2004c). P450scc, adrenodoxin reductase, and steroidogenic factor 1 (SF-1) have also been detected in immortalized sebocytes and sebaceous gland, and the corresponding antigens have been detected *in situ* in human epidermis, hair follicle and

sebaceous gland (Thiboutot et al., 2003). Sebocytes also demonstrated the transformation of 22R-hydroxycholesterol to 17-hydroxypregnenolone (Thiboutot et al., 2003).

3.2. Steroidogenic enzymes in the skin

The skin is known to express the genes for enzymes involved in the sequential metabolism of pregnenolone to corticosteroids including 3 β HSD, cytochromes P450c17 and P450c21, and the MC2-R gene (Dumont et al., 1992; Slominski et al., 1996d; Thiboutot et al., 2003). Actual functional activity for these enzymes has also been demonstrated in cell extracts and cultured skin cells (Dumont et al., 1992; Rogoff et al., 2001; Slominski et al., 2004c). Consistent with these findings, rapid metabolism of progesterone and deoxycorticosterone (DOC) has been shown in rat skin, melanoma cells and immortalized HaCaT keratinocytes (Slominski et al., 1999a; Slominski et al., 2000a; Slominski et al., 2002). In cultured malignant melanocytes, progressive transformation of progesterone to DOC and further metabolism to 18-hydroxy-DOC and corticosterone, but not to aldosterone, has been documented (Slominski et al., 1999a). In HaCaT keratinocytes, progesterone and DOC are metabolized rapidly to steroid products different from corticosterone, aldosterone and cortisol (Slominski et al., 2002).

3.3. Cortisol production in human skin

While these findings already point to mammalian skin as a site of glucocorticoid synthesis, evidence that this actually occurs *in situ* has recently come from the study of microdissected, organ-cultured human scalp hair follicles: their epithelial compartment engages in cortisol synthesis and secretion, which can be stimulated by ACTH and CRH (Ito et al., 2005). Also, corticosterone and cortisol production has been shown by cultured normal human melanocytes and fibroblasts (Slominski et al., 2005e; Slominski et al., 2005f; Slominski et al., 2006c). Therefore, human skin is an extraadrenal site of glucocorticoid synthesis and expresses a steroidogenic pathway suitable for operation in intra-, auto or paracrine modes of action.

4.1. Functionality of the cutaneous HPA axis homolog

By analogy with the central response to systemic stress, we have proposed that the cutaneous response to local stressors is highly organized and mediated by a homolog of the central HPA axis, operating as local coordinator and executor (Slominski and Mihm, 1996; Slominski and Wortsman, 2000; Slominski et al., 2000d; Slominski et al., 2001; Slominski et al., 1999c). Mediators of this axis, i.e., CRH, urocortin and POMC peptides, would then regulate skin pigmentary, immune, epidermal, dermal and adnexal systems (Bohm and Zouboulis, 2004; Ito et al., 2005; Kauser et al., 2006; Slominski, 2005; Slominski, 2006; Slominski et al., 2004b; Slominski and Wortsman, 2000; Slominski et al., 1999c; Zbytek et al., 2004; Zouboulis et al., 2002). Accordingly, exposure to, for example, UV light (physical stress), or biological or chemical stress, would activate pathways involved in the local production of CRH and CRH related peptides and POMC-derived peptides (Slominski et al., 1996b; Slominski et al., 2004b; Slominski and Wortsman, 2000; Slominski et al., 1999c; Zbytek et al., 2006). Signals generated by the integrated actions of these peptides would counteract the local effects of the stressor agent and also attenuate the attendant cutaneous responses (Slominski, 2005; Slominski and Wortsman, 2000; Slominski et al., 2000d). The key tenants of the above hypothesis are now firmly supported by experimental evidence (Ito et al., 2005; Slominski, 2006; Slominski et al., 2005e; Slominski et al., 2005f). For example, incubation of normal epidermal melanocytes and dermal fibroblasts with CRH triggers a functional cascade, which is structured hierarchically along the same algorithm as in the HPA axis: CRH activates CRH-R1; this stimulates cAMP accumulation which increases POMC gene expression and production of ACTH (Slominski et al., 2005f). While this sequence was not detected in cultured, isolated keratinocytes, melanocytes respond to CRH and ACTH with enhanced production of cortisol and corticosterone, an effect abolished by POMC gene silencing or by

the potent CRH-R1 antagonist, antalarmin (Slominski et al., 2005f). Fibroblasts, in turn, respond to CRH and ACTH stimulation with enhanced production of corticosterone, but not of cortisol (which is produced constitutively), with ACTH being more potent as a stimulus than CRH (Slominski et al., 2005f).

The above suggests that melanocytes and fibroblasts display a CRH-led system organized similar to that operating at the systemic level. In the case of fibroblasts, the divergence from the central axis occurs at the most distal step, where corticosterone rather than cortisol is the main steroid stimulated by CRH and ACTH. This pattern suggests differential structure of the response to stress in defined skin cell populations, and analogy in the activation sequences at the single cell and whole body levels. Of interest is the constitutive production of cortisol by cultured dermal fibroblasts, the significance of which remains to be determined (Slominski et al., 2006c). Recent data indicate that the UVB induced production of CRH by human neonatal melanocytes is mediated by the PKA pathway, with sequential involvement of CRH -CRH-R1 in the stimulation of POMC expression (Zbytek et al., 2006).

In organ-cultured, microdissected human anagen hair follicles, CRH treatment upregulates the expression of POMC mRNA, ACTH and α -MSH peptides, CRH-R1 and CRH-R2, and MC1-R and MC2-R (Ito et al., 2005). Moreover, either CRH or ACTH enhanced accumulation of cortisol in keratinocytes of the outer-root sheath of the human-scalp hair follicle, and its secretion into the media (Ito et al., 2005), while hydrocortisone treatment down-regulated CRH-R1 in the hair follicle outer-root sheath, mirroring its classical central feedback regulatory effect (Ito et al., 2005). These findings not only identify human scalp hair follicles as an excellent, clinically highly relevant model for studying a complete, fully functional peripheral HPA axis equivalent system, but also suggest that at least human hair follicle keratinocytes *in situ* are capable of synthesizing and secreting cortisol when their characteristic epithelial-mesenchymal interactions are maintained.

5. Conclusions and Perspectives

Human skin expresses all key elements of the HPA axis, and does so in a differential and highly organized manner, under establishment of cell type-specific, fully functional regulatory loops (Slominski, 2005; Slominski and Wortsman, 2000). Within human skin, the currently available data suggest that neural crest-derived melanocytes and hair follicles display activation sequences and feedback regulation that most closely reproduce key characteristics of the central HPA axis at the peripheral tissue/single cell levels (Ito et al., 2005; Slominski, 2006; Slominski et al., 2005f).

Concerning the CRH signaling system, it is well documented that CRH-transgenic (TG) mice develop significant skin abnormalities (Stenzel-Poore et al., 1992). However, those can be associated with central HPA activation, namely elevated levels of adrenal corticosterone production. Phenotypic analysis of the skin in CRH-R1 and CRH-R2-KO mice indicated a role for the latter receptor in the regulation of hair growth (Vale, 2004), in agreement with the compartment-selective distribution of both receptors and their ligands in human and mouse skin (Slominski, 2006; Slominski et al., 2004a). Additional support for this concept arises from the observation that selective CRH-R2 and CRH-R1 agonists exert differential effects on the growth and pigmentation of human hair follicle (Kausar et al., 2006). In addition, (Yaswen et al., 1999) clearly demonstrated the defect in skin pigmentation in POMC-knockout (POMC-KO) mice. The latter study, however, showed that this effect is dependent on the genotype of the mice used for testing, e.g., in C57BL6/J mice, a pigmentary effect of the POMC-KO is absent, likely due to the fact that this strain is recessive for agouti protein (Slominski et al., 2005a). Other cutaneous abnormalities in POMC-KO mice (including those of hair follicle

cycling and the hair follicle immune system (Paus et al., 2006) still remain to be systematically explored.

In relation to corticosteroidogenic activity of the skin, the CYP11A1-KO has yet to be analyzed by professional pigment cell and hair biologists, perhaps, because such mutation would be incompatible with prolonged survival *ex utero* that would impact adequate analysis of cutaneous phenotype. Nevertheless, the instructiveness of genetically modified mice (TG or KO), in relation to human skin physiology and pathology has significant limits that must be clearly recognized and acknowledged. First, mice are essentially nocturnal species, while human skin is continuously exposed to solar radiation during daytime (reviewed in (Slominski, 2005; Slominski and Pawelek, 1998; Slominski and Wortsman, 2000). Second, there are fundamental differences between rodent and human skin anatomy, histology and physiology (Slominski and Wortsman, 2000); the latter in mice shows dramatic changes coupled to the synchronized switches in hair follicle cycling (reviewed in (Slominski and Paus, 1993; Stenn and Paus, 2001). Third, and more specifically, there are striking differences between the human and murine cutaneous CRH/urocortin systems (reviewed in (Slominski, 2006; Slominski et al., 2004a; Slominski et al., 2001).

Concerning local vs systemic effects of “cutaneous HPA axis”, it is still unclear whether one can expect that their mediators are released into the circulation constitutively or under moderate/weak cutaneous stress taking into consideration low levels of their local production (discussed in (Slominski, 2005; Slominski et al., 2000d). Nevertheless, significant cutaneous stress (such as whole body exposure to high energy UVR) may indeed cause a release of “skin HPA axis” mediators into the circulation; since in humans, the induction by solar radiation of endocrine activity of the skin is well documented, e.g., production of vitamin D3 and its biological effects (Slominski and Wortsman, 2000). Prior to clinical testing of human volunteers, histocultured ex-vivo human skin fragments offers a physiologically highly relevant and instructive basic research tool for further exploring the conditions under which normal human skin may generate and secrete substantial quantities of these mediators into the “circulation”.

Concerning the significance of the cutaneous HPA axis for human skin physiology, it is important to point out that there is indeed clinical evidence showing that humans carrying POMC-mutations have abnormal skin e.g., fair skin and red-hair phenotype (reviewed in (Slominski et al., 2000d) and that clinical studies clearly have demonstrated increased production of POMC transcripts and melanocortin production in the skin of patients exposed to UVB (Schiller et al., 2004), and have demonstrated anti-inflammatory effects of MSH peptides or their melanogenic action (reviewed in (Slominski et al., 2004b; Slominski et al., 2000d). Finally, the demonstration that normal, organ-cultured human scalp hair follicles produce CRH, ACTH, α -MSH and cortisol in a manner that imitates key features of the central HPA axis, and respond phenotypically to stimulation with these intrafollicularly generated (neuro-)endocrine signals (Ito et al., 2004; Ito et al., 2005; Kauser et al., 2006; Slominski, 2006; Slominski et al., 2004a) clearly demonstrates the physiological and clinical relevance of intracutaneously established peripheral HPA axis equivalents.

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