Biochemical and Biophysical Research Communications 440 (2013) 265–270

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

11β-Hydroxysteroid dehydrogenase 1 contributes to the pro-inflammatory response of keratinocytes

Saori Itoi, Mika Terao *, Hiroyuki Murota, Ichiro Katayama

Department of Dermatology, Integrated Medicine, Osaka University Graduate School of Medicine, Japan

ARTICLE INFO

Article history:
Received 10 September 2013
Available online 19 September 2013

Keywords:
11β-Hydroxysteroid dehydrogenase 1
Cortisol
Keratinocyte
Inflammation

ABSTRACT

The endogenous glucocorticoid, cortisol, is released from the adrenal gland in response to various stress stimuli. Extra-adrenal cortisol production has recently been reported to occur in various tissues. Skin is known to synthesize cortisol through a *de novo* pathway and through an activating enzyme. The enzyme that catalyzes the intracellular conversion of hormonally-inactive cortisone into active cortisol is 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1). We recently reported that 11β-HSD1 is expressed in normal human epidermal keratinocytes (NHEKs) and negatively regulates proliferation of NHEKs. In this study, we investigated the role of 11β-HSD1 in skin inflammation. Expression of 11β-HSD1 was induced by UV-B irradiation and in response to the pro-inflammatory cytokines, IL-1β and TNFα. Increased cortisol concentrations in culture media also increased in response to these stimuli. To investigate the function of increased 11β-HSD1 in response to pro-inflammatory cytokines, we knocked down 11β-HSD1 by transfecting siRNA. Production of IL-6 and IL-8 in response to IL-1β or TNFα stimulation was attenuated in NHEKs transfected with si11β-HSD1 compared with control cells. In addition, IL-1β-induced IL-6 production was enhanced in cultures containing 1×10^{-13} M cortisol, whereas 1×10^{-5} M cortisol attenuated production of IL-6. Thus, cortisol showed immunostimulatory and immunosuppressive activities depending on its concentration. Our results indicate that 11β-HSD1 expression is increased by various stimuli. Thus, regulation of cytosolic cortisol concentrations by 11β-HSD1 appears to modulate expression of inflammatory cytokines in NHEKs.

1. Introduction

Glucocorticoids (GCs) are one of the most effective classes of drugs for treating acute and chronic inflammatory conditions. GCs have been used in the clinic for more than 50 years. The major anti-inflammatory mechanism of GCs is repression of inflammatory gene transcription factors, such as nuclear factor-κB and activator protein-1 [1,2]. GCs are also widely used as topical ointments for inflammatory dermatitis, such as atopic dermatitis and contact dermatitis. GCs have anti-inflammatory effects on keratinocytes and on inflammatory cells that infiltrate the skin. GCs also affect proliferation, differentiation, and metabolism of keratinocytes [3].

Cortisol, an endogenous GC in humans (corticosterone in rodents), is released in response to various stressors, including physical injury and psychological stress. Cortisol activity depends on glucocorticoid output from the adrenal gland and is controlled by the hypothalamic–pituitary–adrenal (HPA) axis. Various stressors, such as inflammation, viral infection, or trauma, can stimulate the HPA axis [4]. Extra-adrenal cortisol production in various tissues is also reported [5–11]. Skin cells are also known to synthesize cortisol through a *de novo* pathway and through an activating enzyme [12–24].

The enzyme that catalyzes the intracellular conversion of hormonally-inactive cortisone into active cortisol is 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) [25,26]. This enzyme is expressed in many tissues, with the highest levels found in the liver, lung, adipose tissue, ovaries, and central nervous system [25,27].

Expression of 11β-HSD1 occurs in epidermal keratinocytes, dermal fibroblasts, and root sheath cells of the outer hair follicle [22,23,28,29]. In addition, 11β-HSD1 enzymatic activity has been detected in cultured keratinocytes [29]. We previously reported that 11β-HSD1 negatively regulates cutaneous wound healing by modulating proliferation of keratinocytes and fibroblasts [30]. We also reported that expression of 11β-HSD1 is decreased in benign and malignant tumors of the skin, including squamous cell

Abbreviations: 11β-HSD1, 11β-hydroxysteroid dehydrogenase 1; GC, glucocorticoid; TNBS, 2,4,6-trinitrobenzene sulfonic acid; NHEKs, normal human epidermal keratinocytes; HPA, hypothalamic–pituitary–adrenal; rtPCR, real-time polymerase chain reaction; cDNA, complementary DNA; IL-6, interleukin-6; IL-8, interleukin-8; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SD, standard deviation.

* Corresponding author. Address: Department of Dermatology, Integrated Medicine, Osaka University Graduate School of Medicine, 2-2, Yamada-Oka, Suita-shi, Osaka 565-0871, Japan. Fax: +81 6 6879 3039.

E-mail address: mterao@derma.med.osaka-u.ac.jp (M. Terao).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.065

carcinoma, basal cell carcinoma, and seborrheic keratosis, possibly due to increased proliferation of keratinocytes in these tumors [22]. In addition, expression of 11β-HSD1 increases with age and in photo-exposed dermal fibroblasts [23,31].

In this study, we investigated the association between 11β-HSD1 and skin inflammation, especially in keratinocytes. We found that various stimuli, including cytokines, UV-B irradiation, and the hapten, 2,4,6-trinitrobenzene sulfonic acid (TNBS), increased expression of 11β-HSD1 in keratinocytes. We further found that 11β-HSD1 modulated the response to various pro-inflammatory cytokines.

2. Materials and methods

2.1. Materials

Tumor necrosis factor α (TNFα), interleukin-1β (IL-1β) were purchased from R&D Systems (Minneapolis, Minnesota, USA) and dissolved in 0.1% bovine serum albumin in Phosphate Buffered Saline (PBS). TNBS were purchased from Nacalai Tesque (Kyoto, Japan) and dissolved in PBS.

2.2. Cell culture

Normal human epidermal keratinocytes (NHEKs) were purchased from DS Pharma Biomedical (Osaka, Japan). NHEKs were cultured on type-1 collagen plates (Asahi Techno Glass, Funabashi, Japan) in human keratinocyte serum-free media (DS Pharma Biomedical or Life Technologies). Cortisol and bovine pituitary extract were removed from media 24 h prior to experiments.

2.3. Western blotting

Cell samples were placed at 4 °C in lysis buffer (0.5% sodium deoxycholate, 1% Nonidet P40, 0.1% sodium dodecyl sulfate, 100 μg/ml phenylmethyl sulfonyl fluoride, 1 mM sodium orthovanadate, provanadate, and protease inhibitor cocktail). Thirty micrograms of protein were separated on SDS–polyacrylamide gels and transferred onto polyvinylidine fluoride membranes (Bio-Rad, Hercules, CA, USA). Non-specific protein binding was blocked by incubating membranes in 5% w/v non-fat milk powder dissolved in TBS-T (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, and 0.1% v/v Tween-20). The membranes were incubated with sheep anti-11β-HSD1 antibody (The Binding Site, Birmingham, UK) at a dilution of 1:1000 overnight at 4 °C or with mouse monoclonal anti-β-actin (Sigma–Aldrich, St. Louis, MO, USA) at a dilution of 1:5000 for 60 min at room temperature. Membranes were then washed three times in TBS-T (5 min each time). Finally, membranes were incubated with either HRP-conjugated anti-mouse, or anti-sheep antibody at a dilution of 1:10000 for 60 min at room temperature. Protein bands were detected with the ECL Plus kit (Thermo Scientific, Rockford, Illinois, USA).

2.4. Small interfering RNA (siRNA) transfection

NHEKs (50,000 cells/ml) were seeded on type-1 collagen coated plates 1 day prior to transfection. Cells were transfected with 50 nM 11β-HSD1 siRNA or control siRNA (Invitrogen) using RNAi MAX (Invitrogen). Culture media was replaced 6 h later. Cells were used for experiments 48 h after transfection.

2.5. RNA isolation and quantitative real-time polymerase chain reaction (rtPCR)

Total RNA was isolated from cells with the SV Total RNA Isolation System (Promega). The product was reverse-transcribed into first-strand complementary DNA (cDNA). Expression levels of 11β-HSD1, interleukin-6 (IL-6), and interleukin-8 (IL-8) were measured with the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Levels of mRNA were normalized to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Sequence-specific primers were designed as follow: 11β-HSD1, sense: 5′-tctcctctctggctgggaaag-3′, antisense: 5′-gaacccatccaaagcaaacttg-3′; IL-6, sense: 5′-gaaagcagcaaagaggcact-3′, antisense: 5′-tttcaccaggcaagtctcct-3′; IL-8, sense: 5′-tctgcagctctgtgtgaagg-3′, antisense: 5′-acttctccacaaccctctgc-3′; and GAPDH, sense: 5′-ggagtcaacggatttggtcgta-3′, antisense: 5′-gcaacaatatccactttaccagagttaa-3′. Real-time PCR (40 cycles of denaturation at 92 °C for 15 s and annealing at 60 °C for 60 s) was performed on an ABI 7000 Prism (Applied Biosystems). Samples without reverse transcriptase (negative control) did not show any amplification.

2.6. Enzyme-linked immunosorbent assay (ELISA)

NHEKs (100,000 cells/ml, 100 μl) were seeded in a 96-well type-1 collagen-coated plate. Cells were allowed to attach for 24 h. Media was then changed to basal media, which did not contain cortisol or bovine pituitary extract. Culture media were harvested and stored at −20 °C. The amount of cortisol was measured with the Cortisol EIA kit (Cayman Chemical Company, Ann Arbor, MI, USA). This ELISA kit also cross-reacts with cortisone (less than 2%). Therefore, we measured the absorbance of media containing 10 nM cortisone. We measured the concentrations of IL-6 and IL-8 with the Quantikine Immunoassay (R&D Systems). Each assay was performed according to the manufacturer's protocol.

2.7. Statistical analysis

The data are expressed as means ± standard deviations (SD). The significance of differences was determined by the unpaired Student's *t*-test.

3. Results

3.1. Various stimuli increased expression of 11β-HSD1 in NHEKs

We evaluated expression of 11β-HSD1 in NHEKs in response to various stimuli, including TNFα, UV-B irradiation, and TNBS. TNFα, TNBS, and UV-B irradiation increased 11β-HSD1 mRNA levels in NHEKs (Fig. 1A). In addition, pro-inflammatory cytokines, such as IL-1β and TNFα, increased protein levels of 11β-HSD1 (Fig. 1B). These stimuli also increased cortisol concentrations in the cell culture media of NHEKs, as measured by ELISA (Fig. 1C). These results suggest that expression of 11β-HSD1 is induced by various stimuli in NHEKs.

3.2. Knockdown of 11β-HSD1 by siRNA decreased cortisol levels in media

We knocked down 11β-HSD1 expression and measured cortisol concentrations in cultured media to determine the effects of 11β-HSD1 on cortisol production in NHEKs. Transfection of 11β-HSD1 siRNA reduced mRNA levels of 11β-HSD1, confirming that knockdown was successful (Fig. 2A). Cortisol concentrations significantly increased upon addition of cortisone. However, knockdown of 11β-HSD1 reduced cortisol production (Fig. 2B). These data indicate that 11β-HSD1 is at least partially responsible for regulating cortisol production in NHEKs.

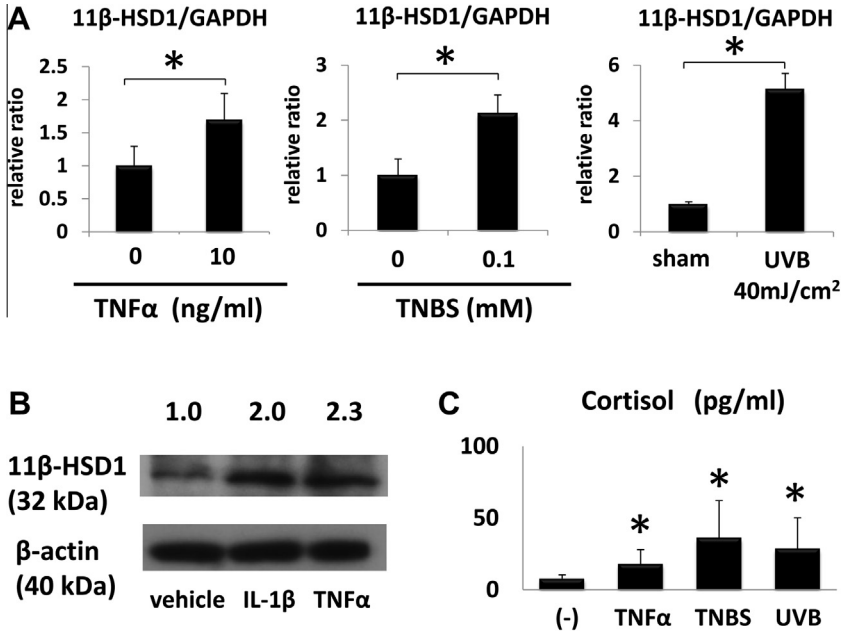

Fig. 1. Various stimuli increased expression of 11β-HSD1 in normal human epidermal keratinocytes (NHEKs). (A) NHEKs were exposed to vehicle, TNFα (10 ng/ml), 2,4,6-trinitrobenzene sulfonic acid (TNBS, 0.1 mM), UV-B irradiation (40 mJ/cm^2), or sham irradiataion, and harvested after 1 hour. Expression of 11β-HSD1 was measured by rtPCR. GAPDH served as an internal control. An asterisk (*) indicates a statistically significant difference (*$P < 0.05$, Student's t-test). (B) NHEKs were stimulated with vehicle, 10 ng/ml IL-1β or TNFα, and Western blotting for 11β-HSD1 was performed after 24 h. β-actin served as an internal control. (C) NHEKs were exposed to vehicle, IL-1β (10 ng/ml), TNFα (10 ng/ml), TNBS (0.1 mM), UV-B irradiation (40 mJ/cm^2), or sham irradiataion for 24 h. Cortisol concentrations in cell culture media were then measured by ELISA.

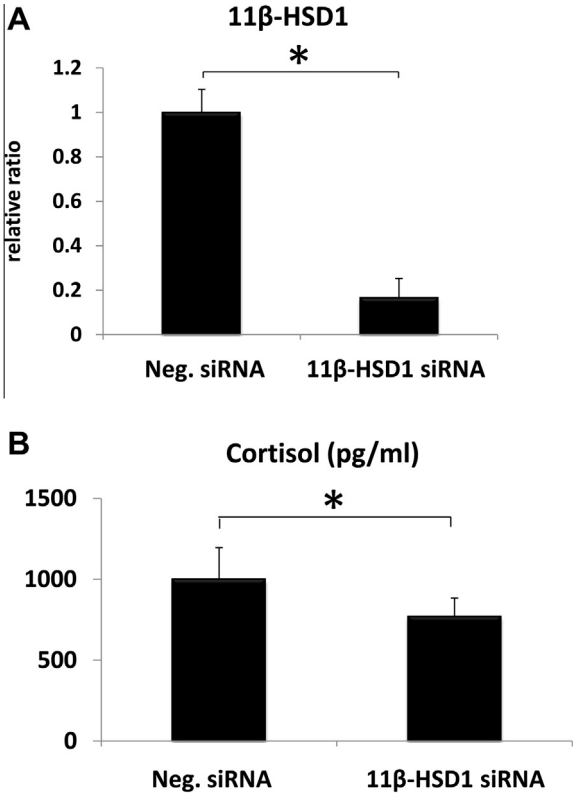

Fig. 2. Knockdown of 11β-HSD1 by siRNA decreased cortisol levels in media. (A) NHEKs were transfected with si11β-HSD1 or control siRNA for 48 h. The mRNA expression of 11β-HSD1 was assessed by rtPCR. GAPDH served as an internal control. An asterisk (*) indicates a statistically significant difference (*$P < 0.01$, Student's t-test). (B) Cortisol concentrations were measured in the media of NHEKs transfected with si11β-HSD1 or control siRNA. Cortisone (1 nM) was added in some experiments. An asterisk (*) indicates a statistically significant difference compared to the vehicle-treated group (*$P < 0.05$, Student's t-test).

3.3. Knockdown of 11β-HSD1 abrogated the response of keratinocytes to pro-inflammatory cytokines

Next, we investigated the role of 11β-HSD1 in the response to pro-inflammatory cytokines. NHEKs were transfected with control siRNA or 11β-HSD1 siRNA and stimulated with IL-1β or TNFα. The mRNA levels of IL-6 were then evaluated. IL-6 expression was induced by IL-1β and TNFα in control siRNA-transfected cells. However, IL-1β- or TNFα-mediated induction of IL-6 was abrogated by knockdown of 11β-HSD1 (Fig. 3A and C). Similarly, IL-1β- or TNFα-induced IL-8 mRNA expression levels were also decreased upon knockdown of 11β-HSD1. In addition, stimulation of control siRNA-transfected cells with IL-1β or TNFα resulted in increased concentrations of IL-6 (Fig. 3E) and IL-8 (Fig. 3F) in culture media. However, these effects were abrogated by 11β-HSD1 knockdown. These results suggest that 11β-HSD1 expression contributes to the production of the inflammatory cytokines, IL-6 and IL-8, in keratinocytes.

3.4. Opposing effects of low-dose versus high-dose cortisol on the inflammatory response of keratinocytes

We examined the effects of cortisol on the pro-inflammatory response of keratinocytes. NHEKs were exposed to various concentrations of cortisol for 24 h and then stimulated with IL-1β. IL-6 mRNA levels were induced by low doses of cortisol (1×10^{-13} and 1×10^{-10} M) in comparison to cells cultured in cortisol-free media. In contrast, mRNA expression of IL-6 was decreased in NHEKs exposed to a higher dose of cortisol (1×10^{-7} M) in comparison to either the lower-dose groups (1×10^{-13} and 1×10^{-10} M) or the control group cultured in cortisol-free media (Fig. 4A). Similarly, IL-6 concentrations in culture media were increased in NHEKs cultured in a low dose of cortisol (1×10^{-10} M) (Fig. 4B).

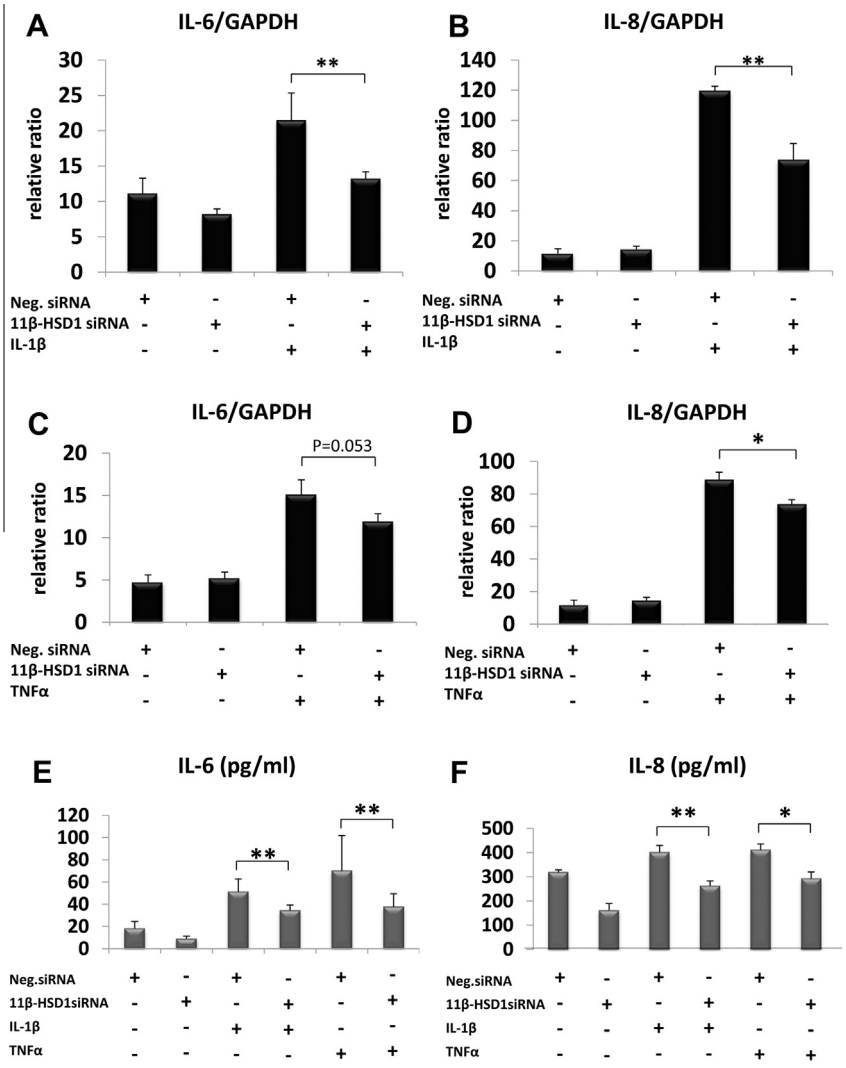

Fig. 3. Knockdown of 11β-HSD1 abrogated the response of keratinocytes to pro-inflammatory cytokines. (A–D) NHEKs were transfected with si11β-HSD1 and stimulated with IL-1β (10 ng/ml) or TNFα (10 ng/ml). Cells were harvested after 1 hour. Expression levels of IL-6 (A and C) and IL-8 (B and D) were measured by rtPCR. GAPDH served as an internal control. An asterisk (*, **) indicates a statistically significant difference (*$P < 0.05$, **$P < 0.01$, Student's *t*-test). (E and F) Keratinocytes were transfected with si11β-HSD1 or control siRNA and stimulated with vehicle, IL-1β (10 ng/ml) or TNFα (10 ng/ml). Culture media were harvested 24 h later. Concentrations of IL-6 (E) and IL-8 (F) were measured by ELISA. An asterisk (*) indicates a statistically significant difference (**$P < 0.01$, *$P < 0.05$, Student's *t*-test).

4. Discussion

In this study, we investigated the role of 11β-HSD1 in NHEKs. We found that expression of 11β-HSD1 was induced by various stimuli. We further revealed that knockdown of 11β-HSD1 diminished the response to pro-inflammatory cytokines. These results suggest that cortisol production, which is mediated by 11β-HSD1, is important for the production of inflammatory cytokines in NHEKs. Thus, 11β-HSD1 expression and activity may be needed in order to achieve an optimal inflammatory response in keratinocytes.

GC medications are well-established systemic and topical anti-inflammatory agents. However, the anti-inflammatory effects of GC-based drugs are achieved at concentrations that are much higher than physiological levels of GCs. Glucocorticoids have the potential to enhance or suppress the immune response depending on the concentration and duration of exposure [32–35]. In fact, 10^{-10} M of corticosterone has been shown to enhance expression of pro-inflammatory cytokines in mouse peritoneal macrophages, whereas higher concentrations of corticosterone (10^{-7} M and 10^{-6} M) suppress the immune response [36]. Furthermore, inactivation of glucocorticoid receptor (GR) in peritoneal macrophages abrogates the immunostimulatory and immunosuppressive actions of GC. Thus, the stimulatory and suppressive effects of GCs on the immune system appear to be mediated through a common pathway involving GR [36]. Low-dose versus high-dose cortisol had opposing effects on the pro-inflammatory response of NHEKs in our experiments. These data are consistent with the opposing actions of cortisol reported in the literature. In addition, these data indicate that the concentration of cortisol is a critical parameter to consider during experimental design and interpretation.

The role of 11β-HSD1 in inflammation has been previously studied. Expression of 11β-HSD1 was induced in the murine macrophage cell line, J774.1, in response to LPS. Importantly, inhibition of 11β-HSD1 significantly suppressed expression of pro-inflammatory cytokines. Thus, 11β-HSD1 mediated pro-inflammatory responses in macrophages [37]. In addition, LPS, TNF-α, and IL-1β augmented expression of 11β-HSD1 in 3T3-L1 preadipocytes. Inhibition of 11β-HSD1 reduced TNFα-induced IL-6, whereas overexpression of 11β-HSD1 further augmented TNFα-induced IL-6 expression [38]. These results are consistent with our findings in NHEKs.

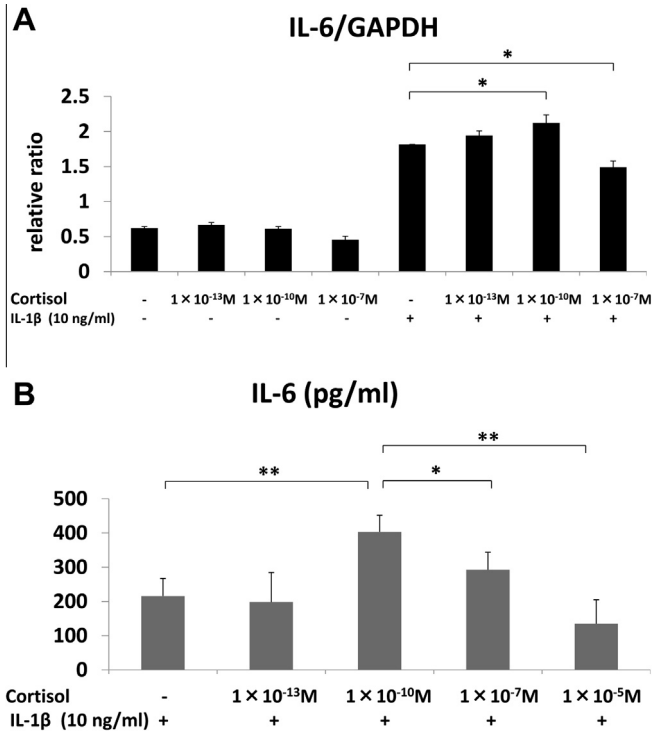

Fig. 4. Opposing effects of low-dose versus high-dose cortisol on the inflammatory response of keratinocytes. (A) The indicated dose of cortisol was added to cultured media for 24 h, and then cells were treated with vehicle or IL-1β (10 ng/ml). Cells were harvested after 1 hour, and IL-6 expression was investigated by rtPCR. GAPDH served as an internal control. (B) The indicated dose of cortisol was added to cultured media for 24 h, and then cells were treated with vehicle or IL-1β (10 ng/ml) for 24 h. Culture media were collected 24 h later, and concentrations of IL-6 were measured by ELISA. An asterisk (*) indicates a statistically significant difference (**$P < 0.01$, *$P < 0.05$, Student's t-test).

Local corticosterone activation by 11β-HSD1 was reported to occur during inflammation *in vivo*. In addition, 11β-HSD1 expression was increased in lung tissue after immune cells were activated by an anti-CD3 antibody [11]. In the K/BxN serum-induced inflammatory arthritis model, mice that were deficient for 11β-HSD1 showed an earlier onset and slower resolution of inflammation than wild-type mice. These data suggest that 11β-HSD1 protects against inflammation in this model of arthritis [39]. These *in vivo* results varied from our results, because11β-HSD1 showed immunosuppressive activities in the *in vivo* model. Investigating local *in vivo* cortisol production in skin is complicated, because local cortisol is produced *de novo* and through 11β-HSD1-mediated pathways. Furthermore, 11β-HSD1 is expressed in other skin cells, including fibroblasts, melanocytes, and immune cells. Thus, in addition to keratinocytes, these cells must be considered as sources of skin cortisol synthesis. To improve current understanding of the function of 11β-HSD1 in keratinocytes, future studies must be performed in keratinocyte-specific 11β-HSD1-knockout mice.

In summary, we demonstrate that 11β-HSD1 modulates a pro-inflammatory response in NHEKs by altering cortisol production. Keratinocytes are located in the outer layer of the skin and are continuously exposed to various mechanical and chemical stimuli. Thus, 11β-HSD1-mediated cortisol production is a potential mechanism by which local skin inflammation occurs.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Exploratory Research (No. 24659529) and a Grant-in-Aid for Young Scientists (A) (No. 24689045). The authors thank Mr. Kenju Nishida, Ms. Yumiko Fujii, Ms. Eriko Nobuyoshi and Ms. Sayaka Matsumura for research assistance.

References

[1] R.M. Sapolsky, L.M. Romero, A.U. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, Endocr. Rev. 21 (2000) 55–89.
[2] I.M. Adcock, G. Caramori, Cross-talk between pro-inflammatory transcription factors and glucocorticoids, Immunol. Cell Biol. 79 (2001) 376–384.
[3] O. Stojadinovic, B. Lee, C. Vouthounis, S. Vukelic, I. Pastar, M. Blumenberg, H. Brem, M. Tomic-Canic, Novel genomic effects of glucocorticoids in epidermal keratinocytes: inhibition of apoptosis, interferon-gamma pathway, and wound healing along with promotion of terminal differentiation, J. Biol. Chem. 282 (2007) 4021–4034.
[4] E.M. Sternberg, Neuroendocrine regulation of autoimmune/inflammatory disease, J. Endocrinol. 169 (2001) 429–435.
[5] M. Noti, N. Corazza, C. Mueller, B. Berger, T. Brunner, TNF suppresses acute intestinal inflammation by inducing local glucocorticoid synthesis, J. Exp. Med. 207 (2010) 1057–1066.
[6] A. Coste, L. Dubuquoy, R. Barnouin, J.S. Annicotte, B. Magnier, M. Notti, N. Corazza, M.C. Antal, D. Metzger, P. Desreumaux, T. Brunner, J. Auwerx, K. Schoonjans, LRH-1-mediated glucocorticoid synthesis in enterocytes protects against inflammatory bowel disease, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 13098–13103.
[7] M. Mueller, I. Cima, M. Noti, A. Fuhrer, S. Jakob, L. Dubuquoy, K. Schoonjans, T. Brunner, The nuclear receptor LRH-1 critically regulates extra-adrenal glucocorticoid synthesis in the intestine, J. Exp. Med. 203 (2006) 2057–2062.
[8] M.J. Young, C.D. Clyne, T.J. Cole, J.W. Funder, Cardiac steroidogenesis in the normal and failing heart, J. Clin. Endocrinol. Metab. 86 (2001) 5121–5126.
[9] K.M. Kayes-Wandover, P.C. White, Steroidogenic enzyme gene expression in the human heart, J. Clin. Endocrinol. Metab. 85 (2000) 2519–2525.
[10] T. Ohtani, T. Mano, S. Hikoso, Y. Sakata, M. Nishio, Y. Takeda, K. Otsu, T. Miwa, T. Masuyama, M. Hori, K. Yamamoto, Cardiac steroidogenesis and glucocorticoid in the development of cardiac hypertrophy during the progression to heart failure, J. Hypertens. 27 (2009) 1074–1083.
[11] N. Hostettler, P. Bianchi, C. Gennari-Moser, D. Kassahn, K. Schoonjans, N. Corazza, T. Brunner, Local glucocorticoid production in the mouse lung is induced by immune cell stimulation, Allergy 67 (2012) 227–234.
[12] N. Ito, T. Ito, A. Kromminga, A. Bettermann, M. Takigawa, F. Kees, R.H. Straub, R. Paus, Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol, FASEB J. 19 (2005) 1332–1334.
[13] A. Slominski, C.E. Gomez-Sanchez, M.F. Foecking, J. Wortsman, Metabolism of progesterone to DOC, corticosterone and 18OHDOC in cultured human melanoma cells, FEBS Lett. 455 (1999) 364–366.
[14] A. Slominski, C.E. Gomez-Sanchez, M.F. Foecking, J. Wortsman, Active steroidogenesis in the normal rat skin, Biochim. Biophys. Acta 1474 (2000) 1–4.
[15] A. Slominski, B. Zbytek, I. Semak, T. Sweatman, J. Wortsman, CRH stimulates POMC activity and corticosterone production in dermal fibroblasts, J. Neuroimmunol. 162 (2005) 97–102.
[16] A. Slominski, B. Zbytek, A. Szczesniewski, I. Semak, J. Kaminski, T. Sweatman, J. Wortsman, CRH stimulation of corticosteroids production in melanocytes is mediated by ACTH, Am. J. Physiol. Endocrinol. Metab. 288 (2005) E701–706.
[17] A. Slominski, B. Zbytek, A. Szczesniewski, J. Wortsman, Cultured human dermal fibroblasts do produce cortisol, J. Invest. Dermatol. 126 (2006) 1177–1178.
[18] A. Slominski, J. Wortsman, R. Paus, P.M. Elias, D.J. Tobin, K.R. Feingold, Skin as an endocrine organ: implications for its function, Drug Discov. Today Dis. Mech. 5 (2008) 137–144.
[19] A. Slominski, J. Wortsman, R.C. Tuckey, R. Paus, Differential expression of HPA axis homolog in the skin, Mol. Cell. Endocrinol. 265–266 (2007) 143–149.
[20] A. Slominski, J. Zjawiony, J. Wortsman, I. Semak, J. Stewart, A. Pisarchik, T. Sweatman, J. Marcos, C. Dunbar, R.C. Tuckey, A novel pathway for sequential transformation of 7-dehydrocholesterol and expression of the P450scc system in mammalian skin, Eur. J. Biochem. 271 (2004) 4178–4188.
[21] A.T. Slominski, M.A. Zmijewski, C. Skobowiat, B. Zbytek, R.M. Slominski, J.D. Steketee, Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system, Adv. Anat. Embryol. Cell Biol. 212 (2012) 1–115 (v, vii).
[22] M. Terao, H. Murota, A. Kimura, A. Kato, A. Ishikawa, K. Igawa, E. Miyoshi, I. Katayama, 11beta-Hydroxysteroid dehydrogenase-1 is a novel regulator of skin homeostasis and a candidate target for promoting tissue repair, PLoS One 6 (2011) e25039.
[23] A. Tiganescu, E.A. Walker, R.S. Hardy, A.E. Mayes, P.M. Stewart, Localization, age- and site-dependent expression, and regulation of 11beta-hydroxysteroid dehydrogenase type 1 in skin, J. Invest. Dermatol. 131 (2011) 30–36.
[24] S. Vukelic, O. Stojadinovic, I. Pastar, M. Rabach, A. Krzyzanowska, E. Lebrun, S.C. Davis, S. Resnik, H. Brem, M. Tomic-Canic, Cortisol synthesis in epidermis is induced by IL-1 and tissue injury, J. Biol. Chem. 286 (2011) 10265–10275.
[25] J.W. Tomlinson, E.A. Walker, I.J. Bujalska, N. Draper, G.G. Lavery, M.S. Cooper, M. Hewison, P.M. Stewart, 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response, Endocr. Rev. 25 (2004) 831–866.

[26] J.R. Seckl, 11Beta-hydroxysteroid dehydrogenases: changing glucocorticoid action, Curr. Opin. Pharmacol. 4 (2004) 597–602.
[27] M.S. Cooper, P.M. Stewart, 11Beta-hydroxysteroid dehydrogenase type 1 and its role in the hypothalamus–pituitary–adrenal axis, metabolic syndrome, and inflammation, J. Clin. Endocrinol. Metab. 94 (2009) 4645–4654.
[28] O. Hennebert, S. Chalbot, S. Alran, R. Morfin, Dehydroepiandrosterone 7alpha-hydroxylation in human tissues: possible interference with type 1 11beta-hydroxysteroid dehydrogenase-mediated processes, J. Steroid Biochem. Mol. Biol. 104 (2007) 326–333.
[29] N. Cirillo, S.S. Prime, Keratinocytes synthesize and activate cortisol, J. Cell. Biochem. 112 (2011) 1499–1505.
[30] M. Terao, S. Itoi, H. Murota, I. Katayama, Expression profiles of cortisol-inactivating enzyme, 11beta-hydroxysteroid dehydrogenase-2, in human epidermal tumors and its role in keratinocyte proliferation, Exp. Dermatol. 22 (2013) 98–101.
[31] A. Tiganescu, A.A. Tahrani, S.A. Morgan, M. Otranto, A. Desmouliere, L. Abrahams, Z. Hassan-Smith, E.A. Walker, E.H. Rabbitt, M.S. Cooper, K. Amrein, G.G. Lavery, P.M. Stewart, 11beta-Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects, J. Clin. Invest. 123 (2013) 3051–3060.
[32] B.S. McEwen, C.A. Biron, K.W. Brunson, K. Bulloch, W.H. Chambers, F.S. Dhabhar, R.H. Goldfarb, R.P. Kitson, A.H. Miller, R.L. Spencer, J.M. Weiss, The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions, Brain Res. Brain Res. Rev. 23 (1997) 79–133.
[33] K.A. Smoak, J.A. Cidlowski, Mechanisms of glucocorticoid receptor signaling during inflammation, Mech. Ageing Dev. 125 (2004) 697–706.
[34] T. Wilckens, Glucocorticoids and immune function: physiological relevance and pathogenic potential of hormonal dysfunction, Trends Pharmacol. Sci. 16 (1995) 193–197.
[35] M.P. Yeager, P.M. Guyre, A.U. Munck, Glucocorticoid regulation of the inflammatory response to injury, Acta Anaesthesiol. Scand. 48 (2004) 799–813.
[36] H.Y. Lim, N. Muller, M.J. Herold, J. van den Brandt, H.M. Reichardt, Glucocorticoids exert opposing effects on macrophage function dependent on their concentration, Immunology 122 (2007) 47–53.
[37] T. Ishii, H. Masuzaki, T. Tanaka, N. Arai, S. Yasue, N. Kobayashi, T. Tomita, M. Noguchi, J. Fujikura, K. Ebihara, K. Hosoda, K. Nakao, Augmentation of 11beta-hydroxysteroid dehydrogenase type 1 in LPS-activated J774.1 macrophages-role of 11beta-HSD1 in pro-inflammatory properties in macrophages, FEBS Lett. 581 (2007) 349–354.
[38] T. Ishii-Yonemoto, H. Masuzaki, S. Yasue, S. Okada, C. Kozuka, T. Tanaka, M. Noguchi, T. Tomita, J. Fujikura, Y. Yamamoto, K. Ebihara, K. Hosoda, K. Nakao, Glucocorticoid reamplification within cells intensifies NF-kappaB and MAPK signaling and reinforces inflammation in activated preadipocytes, Am. J. Physiol. Endocrinol. Metab. 298 (2010) E930–940.
[39] A.E. Coutinho, M. Gray, D.G. Brownstein, D.M. Salter, D.A. Sawatzky, S. Clay, J.S. Gilmour, J.R. Seckl, J.S. Savill, K.E. Chapman, 11Beta-hydroxysteroid dehydrogenase type 1, but not type 2, deficiency worsens acute inflammation and experimental arthritis in mice, Endocrinology 153 (2012) 234–240.