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Targeting β2-adrenergic receptor: a novel approach for limiting psychological stress-induced premature skin aging

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1. Introduction

Psychological stress, exacerbated by contemporary lifestyles, health crises, geopolitical instability, and environmental disruptions, has emerged as a major contributor to chronic physiological dysregulation. Accumulating evidence indicates that stress negatively impacts tissue repair, as demonstrated by impaired wound healing in stressed patients undergoing surgical procedures [1]. Beyond its systemic effects, psychological stress has also been implicated in the acceleration of skin aging, supporting the concept of brain–skin axis [2-4]. Cortisol and epinephrine (Epi, also known as adrenaline) are the main hormonal mediators of stress, with distinct yet complementary biological actions. Cortisol, primarily associated with chronic stress, downregulates the synthesis of extracellular matrix (ECM) components such as collagen and hyaluronic acid, leading to dermal and epidermal thinning clinically described as dermatoporosis [5]. Conversely, Epi is a catecholamine known as a mediator for acute stress. It has rapid systemic effects through the sympathetic-adrenal-medullary (SAM) axis and can also be locally synthesized by epidermal keratinocytes in response to injury [6,7].

Epi exerts its biological effects via its receptors, and especially the β_2-adrenergic receptor (β2-AR), a G protein–coupled receptor expressed by multiple skin cell types, including keratinocytes and fibroblasts [8]. Upon activation, β2-AR signaling modulates the extracellular signal-regulated kinases (ERK) phosphorylation (Figure 1). In keratinocytes, this pathway has been shown to impair cell migration and re-epithelialization, potentially accounting for the delayed wound repair observed in stressed individuals [9,10]. Sustained β2-AR activation also promotes oxidative stress and inflammatory signaling thereby contributing to the age-associated decline in the epidermal regenerative capacity [4,11].

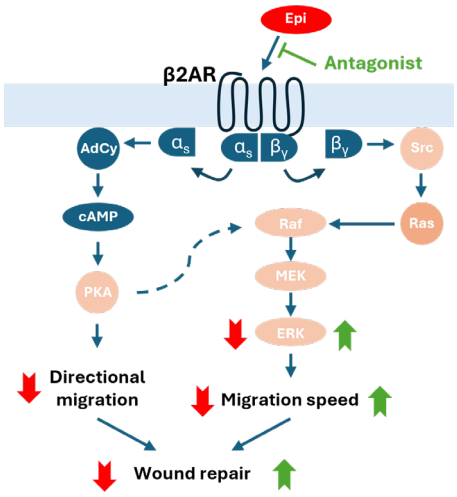

Figure 1 – Epinephrine regulates wound repair in keratinocytes through the β2-AR pathway (adapted from Goodarzi *et al.*, 2021).

While the deleterious effects of Epi on the epidermis are well documented, its impact on dermal architecture and fibroblast function remains poorly characterized [7,9,10]. Nevertheless, β2-AR appears to function as a key molecular transducer of psychological stress in cutaneous tissues, linking neuroendocrine activation to biological processes implicated in premature skin aging [11].

Emerging studies have identified the skin microbiota as a potential modulator of this stress–skin interface. Commensal species such as *Staphylococcus epidermidis* express an aromatic amino acid decarboxylase (SadA), which catalyzes the conversion of aromatic amino acids into trace amines (TAs) that act as ligands for β2-AR to modulate its signaling activity and influence cutaneous stress responses [8,12,13]. These findings reveal a previously unappreciated crosstalk between microbial metabolites and host neuroendocrine pathways. In this context, β2-AR not only integrates psychophysiological inputs but also responds to microbial cues, underscoring its central role in skin homeostasis under stress.

Inspired by this microbiota-host interaction, we generated an amine-based biomimetic compound that mimics TA activity: pyroglutamylamidoethyl indole (pGAEI). We then investigated the impact of Epi on dermal cell function and assessed the efficacy of pGAEI at targeting the β2-AR for preventing stress-mediated adverse effects in the dermis.

2. Materials and Methods

***In silico* docking study**: The molecular structure of the compound was constructed using ChemBioDraw and its geometry optimized via the MM2 force field in ChemBio3D. The crystal structure of the human β2-AR (PDB ID: 6PS6) was retrieved from the Protein Data Bank; water molecules, ions, and co-crystallized ligands were removed using PyMOL. Both ligand and receptor were prepared for docking with AutoDock Tools (v1.5.6), including the addition of polar hydrogens and assignment of Gasteiger charges. Potential binding sites were predicted using DoGSiteScorer, identifying a cavity centered at coordinates (0.87, 7.91, 55.4). A grid box

of 40 × 40 × 40 points with 0.375 Å spacing was defined to encompass the binding site. Molecular docking was performed using AutoDock4 with the Lamarckian Genetic Algorithm, conducting 20 runs to explore ligand conformations. The resulting complexes were analyzed for binding interactions using PyMOL (v2.4.0) for three-dimensional visualization and LigPlot+ (v2.2.9) for two-dimensional interaction mapping.

Cell culture: The immortalized human keratinocyte cell line HaCaT was maintained in Dulbecco's Modified Eagle Medium (DMEM; 1 g/l glucose; Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Biowest SAS, Nuaillé, France), GlutaMAX (Invitrogen), and antibiotics (Invitrogen). Cells were incubated in standard conditions (SC) at 37 °C and 5% CO_2.
Primary normal human dermal fibroblasts (NHDFs) were isolated from breast skin tissue obtained from a 32 y.o. female donor who underwent plastic surgery. Cells were cultured in high-glucose DMEM (4.5 g/l glucose; Invitrogen) supplemented with 10% FBS (Biowest), GlutaMAX, non-essential amino acids (Invitrogen) and antibiotics (Invitrogen), under SC.

Scratch wound assay: HaCaT or NHDF cells were seeded in 6-well plates and cultured to confluence. To inhibit cell proliferation and isolate migratory behavior, cultures were pre-treated with 10 µg/ml mitomycin C (M5353, Sigma) for 1 h at 37 °C, as previously described [7]. A linear scratch was introduced across the monolayer using a sterile SPLScar™ Scratcher (SPL Life Sciences Co., Ltd., Pocheon-si, Gyeonggi-do, South Korea). Wounded monolayers were then incubated with epinephrine (E4250, Sigma) in presence of timolol (a β-adrenergic receptor antagonist) or pGAEI in SC. Cell migration into the wound area was monitored over a 24 h period. Bright-field images were acquired at baseline (T0) and after 24 h using an CKX41 microscope (4× objective) (Olympus Corp., Tokyo, Japan) equipped with a digital camera (Clara Vision, Bièvres, France). For each condition, three representative fields were imaged. The percentage of wound closure was quantified using ImageJ® software (NIH).

ERK phosphorylation assay: Phosphorylated ERK1/2 (pERK) levels were quantified using a fluorescence-based, cell-based ELISA kit (EnzyFluo™ ERK Phosphorylation Assay Kit, EERK-100; BioAssay Systems), according to the manufacturer's instructions. NHDF were cultured in 96-well plates, treated in presence of Epi with timolol or pGAEI for 10 minutes, fixed, and permeabilized *in situ*. Fluorescence was measured using an LB941 Tristar multimode plate reader (Berthold Technologies, Bad Wildbad, Germany) at λ_ex/em = 530/600 nm for pERK and λ_ex/em = 340/470 nm for total protein (reference signal).

Detection of β2-AR and collagen I: NHDF were fixed with PFA (4%), basal β2-AR expression was assessed by immunofluorescence using a primary antibody against β2-AR (NBP2-67187; Novus Biologicals) and an AlexaFluor 594-conjugated secondary antibody (A11037; Invitrogen). For collagen I detection, at the end of the 24 h treatment, cells were fixed and permeabilized with PBS containing Triton X-100, then incubated with a primary antibody against collagen I (1310-01; Southern Biotech), followed by an AlexaFluor 488-conjugated secondary antibody (A11055; Invitrogen). F-actin was stained using rhodamine phalloidine (R415, Invitrogen). In both cases, nuclei were counterstained using a DAPI-containing mounting medium (HP20.1, Roth). Samples were imaged using an Olympus BX60 epifluorescence microscope (40× and 20× objectives respectively for β2-AR and collagen I) and a DP72 camera (Olympus Corporation, Tokyo, Japan). Collagen I fluorescence intensity was quantified with ImageJ® and expressed as the percentage of the field area occupied by the signal.

3D human skin model of psychological stress: Human skin explants were obtained from breast tissue of 22 and 29 y.o. Caucasian females undergoing plastic surgery. Full-thickness 10 mm skin punches were cultured under SC in DMEM (4.5 g/l glucose) supplemented with antibiotics. To mimic systemic psychological stress, explants were treated with Epi in the culture medium. Topical application of active ingredients (20 µl per punch) was performed once daily for 9 consecutive days. At the end of the treatment period, tissues were fixed in PFA (4%) and embedded in paraffin for histological analysis.

Quantification of total collagen in skin sections: Paraffin-embedded sections were stained with picrosirius red to visualize collagen fibers. Sections were imaged using an Olympus BX60 epifluorescence microscope (20× objective). Images were captured with a DP72 camera (Olympus), and three representative regions were acquired per section. Collagen content was quantified using ImageJ® and expressed as the percentage of stained area within a defined region of interest (ROI).

Statistical analysis: Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using JMP software (SAS Institute). Normality of data distribution was assessed using the Shapiro–Wilk test. Baseline homogeneity between groups was evaluated by analysis of variance (ANOVA). Comparisons between treatment groups were made using Student's *t*-test, Welch's *t*-test, or Wilcoxon test, as appropriate based on data distribution and variance. Statistical significance thresholds were defined as follows: (^{ns}) non-significant; $p > 0.05$; (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

3. Results

pGAEI, a TA-mimic with a potential to modulate β2-AR activity

pGAEI is a pseudodipeptide with neuroactive properties [14]. Similarly to the TA produced by the skin microbiota, it also possesses amine moieties that may interact with the β2-AR.

By using an *in silico* docking study, the structure-activity relationship between the TA-mimic and the β2-AR was investigated (Figure 2).

The most favorable binding energy obtained for the ligand-receptor complex is -7.63 kcal/mol, indicating a significant affinity between the molecule pGAEI and β2-AR. The study revealed that the ligand forms hydrogen bonds with residues GLY90 (hydrogen bond length: 2.8 Å), CYS191(1.9 Å), ASN312 (2.1 Å), and TRP313 (1.8 Å). In addition, π-π interactions were observed between the ligand and the PHE193 residue, with interaction distances of 4.7 and 5.0 Å. pGAEI can therefore be considered as a molecule with a chemical structure capable of moderate interactions with the orthosteric pocket of the β2-AR.

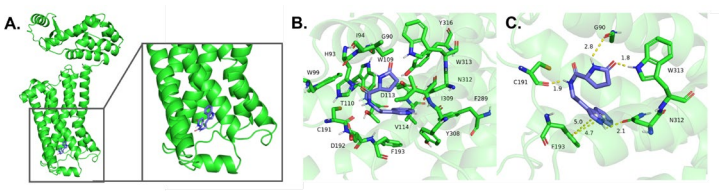

Figure 2 – pGAEI interacts with B2-AR. (A) Representation of pGAEI ligand binding the orthosteric pocket of the β2-AR protein. Analysis of residues (B) of hydrogen bonds and π-π (C) interactions within a 4Å radius around the ligand. pGAEI appears in blue, β2-AR in green.

pGAEI is able to restore the migration of keratinocytes altered by epinephrine (Epi)

Epi is described to alter keratinocyte migration, and that β2-AR antagonists can counteract this effect [7]. The efficacy of pGAEI to restore keratinocyte mobility in the presence of Epi was therefore assessed using a scratch wound assay.

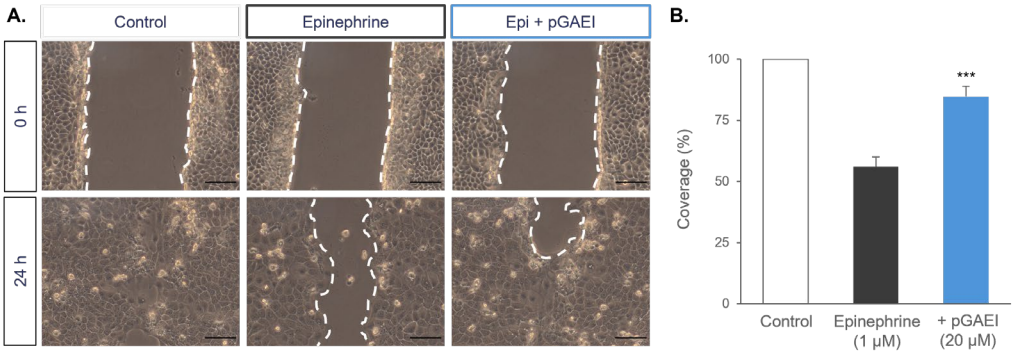

Figure 3 - pGAEI restores keratinocyte migration. Keratinocytes (HaCaT) were exposed to epinephrine (1 µM) for 24 h in the presence or in the absence of pGAEI. Cell migration was observed using a scratch wound assay (A) and quantified (B). Mean ± SEM. *** p-value < 0.001 vs. Epi.

To specifically assess migratory behavior and exclude any contribution from cell proliferation, keratinocytes were pre-treated with mitomycin C to block mitotic activity during the assay. Exposure to 1 µM Epi significantly impaired keratinocyte migration resulting in a 44% reduction

in wound closure at 24 h compared to untreated controls. The treatment with the TA-mimic pGAEI restored keratinocyte migratory capacity (Figure 3).

Fibroblasts (NHDF) express the β2-AR and are affected by epinephrine

The NHDF used in our study were found to express the β2-AR, as revealed by immunostaining analysis (Figure 4A). This confirms that these cells may be direct targets of Epi and may actively contribute to stress-induced alterations of ECM dynamics and regenerative processes. We next investigated the functional consequences of β2-AR activation in dermal fibroblasts by assessing their migratory capacity in response to Epi.

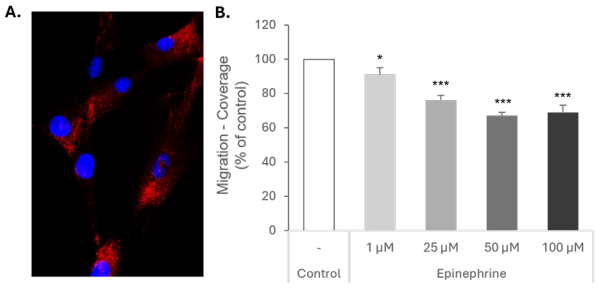

Figure 4 – Epinephrine inhibits fibroblast migratory properties. (A) Immunofluorescence picture of fibroblasts. β2-AR appear in red, nuclei in blue (DAPI). (B) Quantification of the migratory properties of NHDF exposed to increasing concentrations of epinephrine for 24 h using a scratch test assay. Mean ± SEM. * p-value < 0.05, *** p-value < 0.001 vs control.

Epi exerts a concentration-dependent inhibitory effect on fibroblast migration (Figure 4B). In contrast to its effects on keratinocytes, at a concentration of 1 µM, Epi only slightly affects fibroblast migration. The maximal reduction in fibroblast migration was observed from 50 µM and reaches the same range as the one obtained in keratinocytes at 1 µM (Figure 3). This concentration was therefore used for the following 2D experiments performed on fibroblasts.

These findings indicate that dermal fibroblasts are also susceptible to Epi-induced impairment in migration, confirming the contribution of the β2-AR signaling pathway to delayed wound healing and impaired tissue regeneration in stress-exposed skin.

Timolol, a β2-AR antagonist, and pGAEI are able to restore fibroblast migration capacity

In keratinocytes, Epi inhibits cell migration via a β2-AR/ERK-dependent pathway (Figure 1). We therefore assessed the ability of timolol and pGAEI to restore fibroblast migration and analyzed the phosphorylation status of ERK1/2, a key downstream effector of β2-AR signaling (Figure 5).

While exposure to Epi (50 µM) reduced fibroblast migration by approximately 32% compared to untreated control cells, the treatments with either timolol (50 µM) or pGAEI (20 µM) negated this effect by 81% and 69% respectively (Figure 5A,B). These results were consistent with the observation for phospho-ERK. Indeed, Epi inhibited ERK phosphorylation while timolol and pGAEI negated this effect (Figure 5C).

These results further support the central role of the β2-AR/ERK signaling pathway in mediating the deleterious effects of stress hormones on dermal cell behavior. They further highlight the potential of both receptor antagonists and TA-mimetic compounds as effective strategies to preserve the skin regenerative functions under stress conditions.

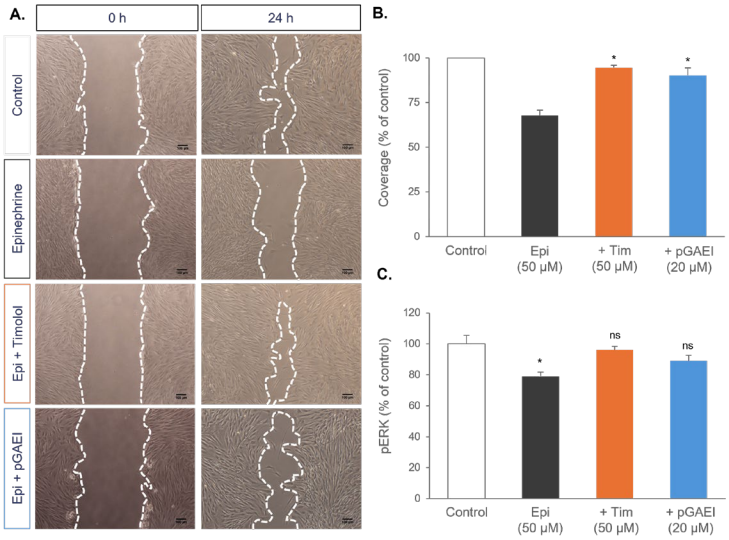

Figure 5 – pGAEI restores fibroblast migration. NHDF were exposed to epinephrine (50 µM) for 24 h in the presence or in the absence of timolol or pGAEI. Cell migration was observed using a scratch wound assay (A) and quantified (B). Mean ± SEM. n=3. * p-value < 0.05 vs Epi. (C) Quantification of phospho-ERK. Mean ± SEM. * p-value < 0.05 vs Control.

pGAEI negates Epi-induced collagen I inhibition in a β2-AR-dependent pathway

While the impact of Epi on fibroblast migration was established, we investigated its effect on ECM remodeling and assessed whether a β2-AR antagonist could reverse these effects. NHDF exposed to Epi (50 µM) displayed a 39% inhibition of collagen I synthesis. This effect was fully prevented by a treatment with timolol. pGAEI (40 µM) also reversed the inhibitory effect of Epi and leads to a 67% increase compared to Epi-treated cells (Figure 6A,B).

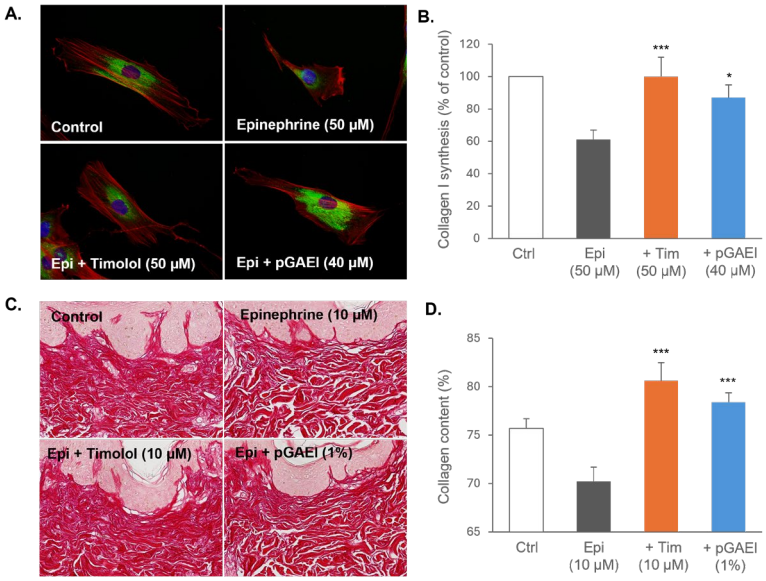

Figure 6 – pGAEI restores collagen synthesis. NHDF and human skin explants were exposed to Epi (for 24 h and 9 days respectively) in the presence or in the absence of timolol or pGAEI. Collagen I synthesis by NHDF was observed (A) and quantified (B). Collagen I appears in green, actin in red and nuclei in blue. Collagen fibers in human skin explants were observed (C, in red) and quantified (D). Mean ± SEM. n=3 or 9. *p-value <0.05; ***p-value <0.001 vs Epi.

These results were further confirmed *ex vivo* by exposing human skin explants to Epi for 9 days. A dramatic decrease in collagen density in the dermis was observed, and both timolol and pGAEI completely negated this alteration (Figure 6C,D).

Altogether, these results support the role of β2-AR signaling pathway in modulating collagen homeostasis under stress conditions, and highlight the potential of TA-mimic compounds as bioactive agents capable of preserving ECM integrity.

4. Discussion

Psychological stress has long been recognized as a significant factor contributing to accelerated aging. As the body's primary interface with the external environment, skin is particularly affected by the physiological responses to stress. As previously noted, stress mediators such as cortisol and epinephrine (Epi) influence skin function and regeneration, thus leading to impaired wound healing, reduced collagen production, and skin aging [2,11]. In this study, we investigated the impact of Epi on skin cells and the potential of a trace amine (TA)-mimic to counteract the negative effects of stress on skin regeneration.

We confirmed that Epi impairs not only keratinocyte migration but also fibroblast migration. This effect is mediated through the β_2-adrenergic receptor (β2-AR) and the downstream ERK signaling pathway. Exposure to Epi resulted in a significant reduction in collagen I production and an increased MMP-1 production by fibroblasts (data not shown), thus contributing to skin aging [11,16]. Importantly, we showed that pGAEI, a TA analogue, can modulate the response to Epi by interacting with the β2-AR/ERK pathway, highlighting a potential therapeutic strategy for mitigating stress-induced skin damage.

Epi, produced during psychological stress or locally in response to skin injury, has long been associated with delayed wound healing. While its impact on keratinocyte migration is well documented [7,9,10], its effects on fibroblast migration remain more contentious. At low, physiological concentration (1 µM), Epi can promote fibroblast migration and proliferation. However, at higher concentrations (≥50 µM; observed during an acute stress), Epi significantly inhibits fibroblast migration, which may exacerbate the slowing of wound healing [11,15]. Our findings align with this dual role of Epi, confirming that high Epi concentrations result in impaired human fibroblast migration and that β2-AR antagonists, such as timolol, can restore fibroblast migration capacity [15,16].

While fibroblasts do not produce catecholamines, they express β2-AR [8]. Our results suggest that this receptor could play a central role in mediating Epi-induced effects in fibroblasts, as evidenced by the restoration of fibroblast migration using timolol, a β2-AR antagonist. This supports the hypothesis that, similarly to keratinocytes, the effects of Epi on fibroblasts are mediated through β2-AR activation [7].

Given the central role of ERK in fibroblast activation and dermal remodeling, and its known involvement in collagen I synthesis via PDGF-induced MAP kinase signaling [17], we further investigated collagen I levels following ERK phosphorylation. Beyond impairing fibroblast migration, Epi exposure also significantly reduces collagen I synthesis by fibroblasts. While this reduction in collagen I production contributes to the observed signs of skin aging and dermal degeneration, it cannot be the sole responsible. In fact, matrix metalloproteinases (MMPs)-induced collagen degradation is another likely mechanism as it was reported that psychological stress may promote their expression [11,16]. These findings provide further evidence that stress not only compromises epidermal regeneration but also disrupts dermal remodeling, thus accelerating the aging process.

In support of these findings, recent research has highlighted the role of microbiota in modulating skin responses to stress. Specifically, the commensal skin bacteria *Staphylococcus epidermidis* can produce postbiotics, including trace amines (TAs), that interact with β2-AR to potentially counteract the detrimental effects of Epi [13]. These TAs, derived from aromatic amino acids, have been shown to modulate β2-AR signaling and may offer a novel approach for mitigating stress-induced skin damage [8].

The docking study revealed that pGAEI exhibits a moderate binding affinity to the β2-AR, forming typical hydrogen bonds, as well as π-π interactions, indicating a stable occupation of the orthosteric site. This moderate affinity suggests a potential for topical dermocosmetic applications, offering targeted modulation of β2-AR pathway.

Taken together, this data suggests that TA-mimics can effectively counteract the adverse effects of psychological stress on skin regeneration. The results provide compelling evidence that targeting the β2-AR with TAs or their mimics represents a promising strategy for combating the signs of stress-induced skin aging.

5. Conclusion

In conclusion, our findings support the idea that psychological stress accelerates skin aging by impairing both epidermal and dermal regeneration processes, with Epi playing a pivotal role in these effects. By targeting β2-AR with a "TA-mimic," we demonstrate the potential to counteract the negative impact of stress on skin health. This approach could be applied more broadly as a pro-regenerative strategy for conditions where psychological stress impairs skin regeneration, such as in wound healing and hair growth.

As a future perspective, it would be beneficial to further explore the combined effects of cortisol and Epi, the two main stress hormones, to provide a more comprehensive understanding of how psychological stress affects skin. Such research could lead to the development of holistic skincare solutions that mitigate the impact of stress on skin health and promote more effective rejuvenation strategies.

6. References

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