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A Novel Role of Endogenous Corticotropin-releasing Hormone (*Crh*) on Dermal Fibroblast Function

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INTRODUCTION

Hypothalamic CRH, a major mediator of the stress response, is involved in the inflammatory response by exerting indirect anti-inflammatory effects via stimulation of glucocorticoid release as well as potent direct proinflammatory effects in a plethora of tissues, including skin. The latter is further supported by that, CRH and its receptors are expressed in human and murine skin. In the process of wounded skin repair, fibroblasts from the wound edges migrate into the wound and proliferate in order to fill the site of the wound. This process is highly coordinated and mediated by locally released growth factors and cytokines which likely act in an autocrine/paracrine manner. IL-6 is a pro-inflammatory cytokine critically involved in cutaneous wound healing. We have previously shown that CRH regulates IL-6 expression during inflammation and that *Crh* deficient mice have accelerated wound healing in vivo and suppressed tissue IL-6 expression. Based on the above, *the aim of our study* was to clarify the role of endogenous CRH on the function of primary dermal fibroblasts, *in vitro*.

METHODS

Primary fibroblasts were isolated from the skin of wild type (*Crh+/+)* and *Crh* deficient (*Crh-/-*) neonatal mice as well as from human foreskin. Evaluation of the proliferation rate of all cells was performed using the MTT and the thymidine incorporation methods. Apoptotic cell death was measured with FACS analysis. Cytokine levels were measured with ELISA (murine cells) or with Chemiluminescence (human cells). Evaluation of the migration rate was performed using the scratch assay. The presence and the functional role of CRF receptors in dermal fibroblasts was tested with RTPCR and cAMP accumulation assays, respectively.

RESULTS

Our results showed that *Crh-/-* fibroblasts have significantly compromised IL-6 and TGF-β secretion compared to that of *Crh*+/+ mice. Treatment of cells with CRH (10 nM) did not affect the secretion of either IL-6 or TGF-β in both genotypes. Furthermore, *Crh-/-* fibroblasts had significantly higher basal proliferation rate compared to *Crh*+/+ fibroblasts. Treatment with CRH (10 nM) had no effect on the proliferation rate of cells of either genotype. No difference was observed in apoptosis between the two genotypes. Furthermore, the number of *Crh-/-* cells migrated into the wounded area was significantly higher than that of *Crh*+/+ cells in the in vitro wound assay.

Treatment of cells of either genotype with CRH did not alter their migration rate. Finally, in order to examine the effect of CRH on human fibroblasts, experiments using the CRF receptor antagonists, antalarmin and a-helical CRF(9-41) were performed. These experiments revealed increased proliferation and migration rate and suppressed IL-6 secretion of CRF_1 antagonist-treated fibroblasts.

DISCUSSION

In summary, our *in vitro* and *in vivo* findings on the role of CRH in–cutaneous wound healing demonstrate accelerated repair in the *Crh-/-* mice, possibly due to the increased migration and proliferation rate of the *Crh-/-* dermal fibroblasts. Furthermore, the reduced secretion of IL-6 by *Crh-/-* fibroblasts, in conjunction with the interrelated roles of IL-6 and CRH and the presence of CRH and its receptors in the skin, underlines the significance of locally acting CRH in the biology of these cells. Our findings support the direct effects of CRH in skin biology and provide interesting insights for the potential usefulness of the developing specific agonists and antagonists of the CRH family in dermal injury or other skin diseases.