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Cultured Human Dermal Fibroblasts do Produce Cortisol

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TO THE EDITOR

Known steroidogenic functions of the skin that are represented by the synthesis and metabolism of androgens and estrogens (Chen *et al.*, 2002; Zouboulis and Degitz, 2004) probably include corticosteroids (Slominski *et al.*, 2000; Slominski, 2005). Thus, the skin expresses both the genes and proteins involved in the synthesis of adrenal corticosteroids (Slominski *et al.*, 1996, 1999, 2004; Rogoff *et al.*, 2001). Metabolism of progesterone to deoxycorticosterone and corticosterone has been documented in melanoma cells and in rat skin (Slominski *et al.*, 1999, 2000); corticotropin-releasing hormone- and ACTH-regulated cortisol production was demonstrated in histocultured human hair follicles (Ito *et al.*, 2005); and corticotropin-releasing hormone and ACTH enhancement of cortisol production was demonstrated in epidermal melanocytes (Slominski *et al.*, 2005b). In contrast, in human dermal fibroblasts cortisol production was not enhanced by corticotropin-releasing hormone and ACTH (there was only selective stimulation of corticosterone production; Slominski *et al.*, 2005a). However, since the cortisol-like immunoreactivity was detected by ELISA, we investigated the chemical nature of the antigen.

Normal human adult dermal fibroblasts (from lightly pigmented female: lot 1C0317 (Cascade Biologics Inc., Portland, OR)) were cultured in Epilife medium with EpiLife Defined Growth Supplement free of serum (Cascade Biologics). The cells were washed twice with phosphate-buffered saline before incubation in supplement-free EpiLife medium with progesterone (10^{-6}M), ACTH (10^{-}M, but added again after 12 h), and/or IBMX (phosphodiesterase inhibitor) (10^{-}M). After 24 hours, media were extracted for cortisol identification and quantification as described previously (Slominski *et al.*, 2005b). Cortisol in supernatants was measured with ELISA (Bio-Quant Inc., San Diego, CA), whereas chemical nature of the compounds was assessed by liquid chromatography mass spectrometry analysis with auto-mass spectrometry/mass spectrometry as described previously (Slominski *et al.*, 2005b). Data were analyzed using Prism 4.00 (GraphPad Software), and is presented as mean + SEM ($n = 4$).

Testing dermal fibroblasts for the release of cortisol into the media showed that addition of progesterone (10^{-}M) stimulated significantly (4.3-fold) cortisol production; further enhancement (2.5 times) was observed after the addition of 10^{-}M IBMX (phosphodiestearse inhibitor) but not ACTH (11-fold increase over the basal values obtained from unstimulated cells; Figure 1). The addition of corticotropin-releasing hormone did not affect the production of cortisol (not shown). Liquid chromatography mass spectrometry analyses of extracted steroids showed the presence of an $[M + H]^+$ ion at m/z 363 (real mass 362) with retention time of 11 minutes identical to the cortisol standard (Figure 2). Final confirmation of the product

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identity as cortisol was obtained with mass spectrometry/mass spectrometry analysis that yielded the same fragment ions as those of the cortisol standard: $m/z = 345$ ($[M + H]^+ - H_2O$), $m/z = 327$ ($[M + H]^+ - 2H_2O$), and $m/z = 309$ ($[M + H]^+ - 3H_2O$) (Figure 2).

These data document the capability of human dermal fibroblasts to produce cortisol molecules identified by rigorous analysis with liquid chromatography mass spectrometry[2]. Moreover, and similar to normal (Slominski *et al.*, 2005b) and malignant melanocytes (Slominski *et al.*, 1999), fibroblasts also displayed a precursor substrate relationship in the accumulation of cortisol, for example, the addition of progesterone-stimulated production of the final corticosteroid cortisol, which was further enhanced by a phosphodiesterase inhibitor (IBMX). Nevertheless, in contrast to melanocytes ACTH and corticotropin-releasing hormone did not stimulate cortisol production in fibroblasts, related, perhaps, to defective coupling of MC-2R (if expressed) to intracellular signaling of the glucosteroidogenic pathway in cultured fibroblasts. Thus, these studies extend the *ex vivo* finding of cortisol production by hair follicles (Ito *et al.*, 2005), adding an important component of heterogeneity to the pleiotropic expression of hypothalamic-pituitary-adrenal mediators in the skin (Slominski and Wortsman, 2000; Slominski, 2005). The role of these factors in the cutaneous response to stress remains to be fully characterized.

Acknowledgements

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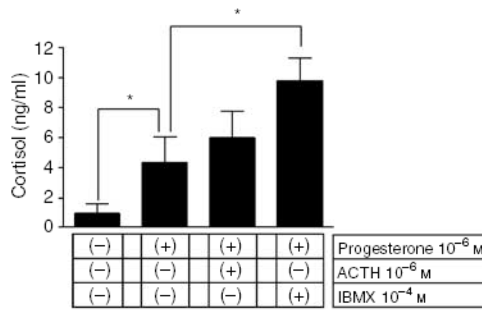

Figure 1. Effect of progesterone on cortisol synthesis

Fibroblasts were cultured in 24-well plates seeded at density of 10^5 cells/well. Differences between control and experimental treatments: *$P<0.05$.

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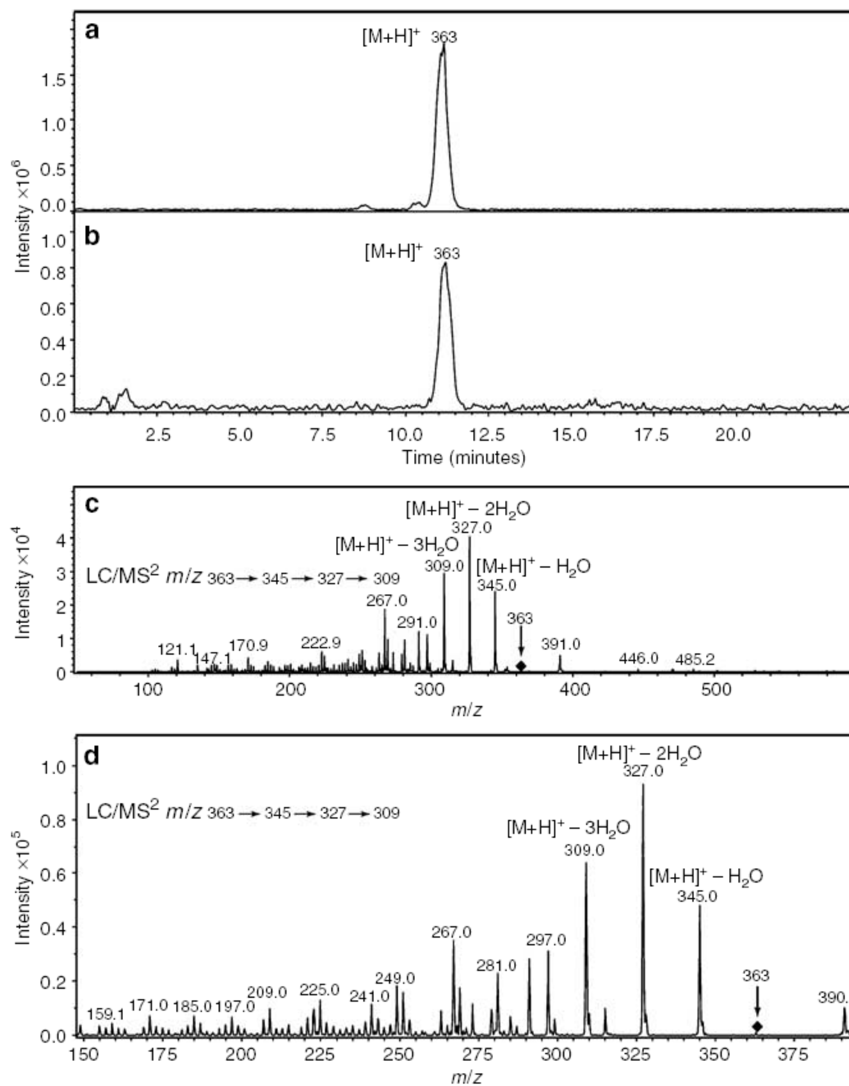

Figure 2. Liquid chromatography mass spectrometry (LC/MS^2) identifies cortisol in cultures of fibroblasts

Fibroblasts were cultured in Petri dishes seeded at density of 2×10^6 cells/dish. (**a**, **c**) Cortisol standard and (**b**, **d**) extracts from conditioned media.