Vol. 189, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1992 Pages 72-78

PRODUCTION OF CATECHOLAMINES IN THE HUMAN EPIDERMIS

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Received October 7, 1992

Cell-free extracts from human full thickness skin (i.e.,epidermis and dermis), suction blister roofs (i.e., epidermis) and from human keratinocytes express biopterin-dependent tyrosine hydroxylase a well as phenylethanolamine-N-methyl transferase, both representing key enzymes for the biosynthesis of epinephrine. These enzyme activities could not be detected in cell extracts from human melanocytes and human fibroblasts. Since keratinocytes in the human epidermis, and in cell cultures, express a high density of beta-2-adrenoceptors, and this signal transduction system regulates intracellular calcium homeostasis, it can be concluded that epinephrine production in the epidermis activates calcium transport via the beta-2-adrenoceptor system. Our results show for the first time that the human epidermis has the capacity to independently produce epinephrine.

Human keratinocytes, under *in vitro* conditions, have been shown to express a high density of beta-2-adrenoceptors with approximately 7000 receptors/cell, meanwhile, alpha and beta-1-adrenoceptors are absent as shown by functional studies and by radioligand binding *in vitro* (1 - 6). These cells at all stages of their development from the basal layer up to the granular layer, contain an even higher density of beta-2-adrenoceptors compared to human heart muscle (3). It is well established that myocardial cells increase the intracellular free calcium concentration and activate the adenylate cyclase/cyclic AMP cascade upon beta-2-adrenergic stimulation (3). Koizumi *et al.* (7) were the first to report that human keratinocytes increase their intracellular calcium concentration via stimulation of the beta-2-adrenoceptor signal by epinephrine. Using isotopic labelled ^{45}calcium, it has been demonstrated that increases in intracellular calcium

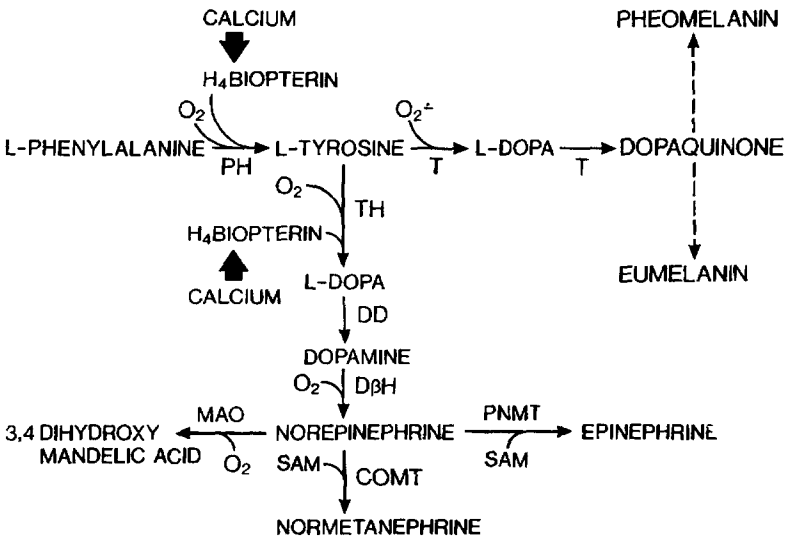
0006-291X/92 $4.00

concentration in different cell lines of human keratinocytes and in human melanoma cells, depends on the beta-2-adrenoceptor density (6, 8). High concentrations of extracellular calcium in the culture medium of human keratinocytes caused a significant down-regulation of beta-2-adrenoceptor expression (4). Since calcium has many functions in the human epidermis regulating keratinocyte growth, differentiation, and desmosome formation as well as melanocyte growth, differentiation, adhesion and melanin biosynthesis (9-14); these results underline the importance of beta-2-adrenoceptor expression in the regulation of calcium homeostasis in the human epidermis. Increases in intracellular calcium have also been demonstrated in human skin under inflammatory conditions triggered by the production and release of tumor necrosis factor α (TNF α) from keratinocytes (15, 16).

Until recently, it has been assumed that epinephrine in human skin originates from the adrenal glands and the sympathetic free nerve endings (17). Experiments with cell extracts from full thickness rat skin revealed the presence of an extra-neuronal phenylethanolamine-N-methyl transferase (PNMT) for epinephrine biosynthesis from nor-epinephrine and S-adenylmethionine (SAM) (18). PNMT has also been detected in psoriatic human skin by immunohistochemistry with an antibody to this enzyme (19). To our knowledge, both groups did not identify the specific cells involved in PNMT activity in the epidermis (18, 19). We were now able to show that human keratinocytes, representing the major cell population of the human epidermis express two key enzymes of the catecholamine biosynthetic pathway: (a) biopterin-dependent tyrosine hydroxylase (TH) and (b) phenylethanolamine-N-methyl transferase (PNMT).

MATERIALS AND METHODS

TH activity was measured by following the rate of exchange of ^3H_2O from 3,5 ^{3}H-labelled L-tyrosine in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) as electron donor and biopterin as cofactor (19). Using keratinocyte cell-free extracts, the ^3H_2O exchange from radiolabelled L-tyrosine yielded stoichiometry upon elution of ^3H_2O from Dowex 50 H^+/borate columns (20). The TH activity towards ^{3}H-labelled-L-tyrosine confirmed this reaction as the major metabolic pathway in the cytosol fraction of these cells, whereas TH activity could not be detected in either human melanocytes or human fibroblasts. In human melanocytes and melanoma cells, the principal activity for L-tyrosine metabolism was catalyzed by tyrosinase (Scheme I). By contrast to experiments with human keratinocytes, cytosol extracts prepared from epidermal suction blister roofs, and from full thickness skin, contained significant tyrosinase activity. Since both tyrosinase and TH catalyze the exchange of ^3H_2O from 3,5 ^{3}H-labelled-L-tyrosine, yielding L-dopa as the common intermediate, TH activity could only be determined by subtraction of tyrosinase activity (i.e. 3,5 ^{3}H L-tyrosine plus cell extracts alone) from the total ^3H_2O exchange activity (i.e. 3,5 L-tyrosine plus cell extracts plus NADPH plus biopterin). Phenylethanolamine-N-methyl transferase activities (PNMT) were determined by following the formation of ^{3}H methyl-labelled epinephrine from norepinephrine and ^{3}H methyl-S-adenosyl-L-methionine (6, 18).



Scheme I.
The melanins and epinephrine are synthesized by the activities of L-phenylalanine hydroxylase yielding L-tyrosine as the common intermediate. Tyrosinase (T) converts L-tyrosine to dopaquinone in a 2-step reaction and dopaquinone is a common substrate for the synthesis of eumelanin (black) and pheomelanin (red). Tyrosinase prefers superoxide anion radical as a substrate (O_2^-) over O_2 (24). Tyrosine hydroxylase (TH) uses O_2 as a substrate and requires NADPH/Biopterin to produce the essential cofactor tetrahydrobiopterin; this step is regulated by intracellular calcium. L-dopa is then converted to dopamine by dopadecarboxylase (DD) and dopamine is oxidized to norepinephrine by dopamine β hydroxylase (DβH). Norepinephrine concentration is regulated by monoamine oxidase (MAO) and catechol-o-methyl transferase (COMT). Phenylethanolanine-N methyl transferase (PNMT) catalyzes the synthesis of epinephrine from norepinephrine and S-adenosyl-L-methionine (SAM).

RESULTS

Figure 1 presents a comparison of the specific activities of TH in cell extracts from human full thickness skin (FTS), human epidermal suction blister roofs (E), human keratinocytes (K), human melanocytes (M) and human fibroblasts (F). These results unambiguously show that TH activity is located in the cytosol fraction of human keratinocytes. Figure 2 demonstrates that tetrahydrafolic acid (THF) can replace NADPH and biopterin as a less efficient electron donor/cofactor for TH. The activity with THF is not inhibited by 10^{-3} M calcium. However, when NADPH and biopterin are used as the electron donating system, the addition of 10^{-3} M calcium inhibited TH activity more than 90%. This result indicates that calcium inhibits tetrahydrobiopterin synthesis from NADPH and biopterin.

Cell-free extracts from human full thickness skin (FTS), epidermal suction blister roofs (E) and human keratinocytes (K) contain an extremely active PNMT (Figure 3). The high specific activities for the biosynthesis of epinephrine from norepinephrine support a major activity for

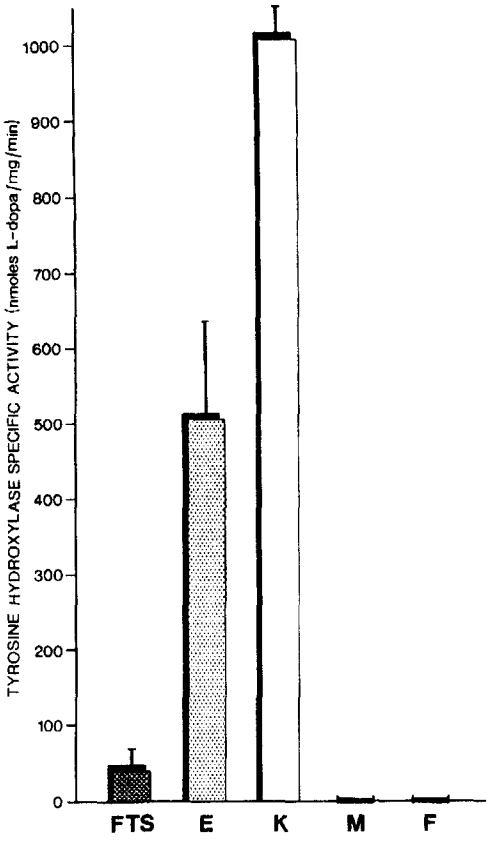


Figure 1.
Tyrosine hydroxylase activities were determined in duplicate in the cytosol fraction of full thickness skin (FTS), epidermal suction blister roofs (E), keratinocytes (K), melanocytes (M), and fibroblasts (F). Assays were performed in 1.0 ml tris-HCl buffer (0.05 M) at pH 7.5 containing 3,5 ^{3}H-labelled L-tyrosine (72.5 Ci/mM) (4.6 µmoles), NADPH (20 µmoles) and biopterin (20 µmoles) together with duplicate tyrosinase controls without NADPH and biopterin. All reactions were started by the addition of 100 µl cell extract. Specific activities were determined from the reaction rates for $^{3}H_2O$ exchange (19) as (nmoles L-dopa formed/mg of protein/minute).
Human keratinocytes were established in serum-free MCDB-153 medium with 0.1 x 10^{-3} M calcium.
Human fibroblasts were grown in DMEM medium with 10% fetal calf serum.
Human melanocytes were established in (a) HAMS-F10 medium with 1% Ultraser G and (b) in MCDB 153 with 10% fetal calf serum without TPA and cholera toxin. Both cell lines from Amsterdam and Newcastle showed no tyrosine hydroxylase activity.

keratinocytes since no PNMT activity could be detected in human melanocytes and human fibroblasts (Figure 3).

DISCUSSION

Earlier results for PNMT activity measured on human full thickness skin showed that epinephrine is rapidly synthesized and degraded (6). Both catechol-o-methyl transferase (COMT) and monoamine oxidase (MAO) have been previously reported to be present in human

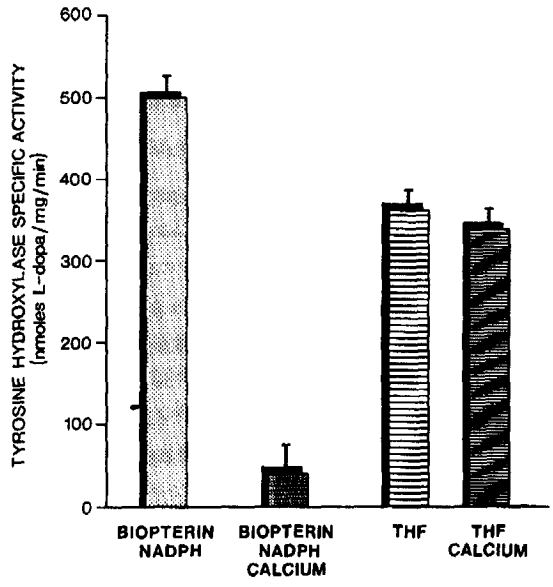


Figure 2.
Duplicate assays were performed for TH activities on cell extracts from epidermal suction blister roofs with Biopterin plus NADPH, Biopterin plus NADPH plus Calcium (10^{-3} M), tetrahydrofolic acid,THF, and tetrahydrafolic acid plus Calcium (10^{-3} M).

keratinocytes, melanocytes and melanoma cells, indicating that these well established degradation pathways for norepinephrine are available (22, 23). However, if one considers that norepinephrine stimulates alpha and beta-1-adrenoceptors, and these receptors are absent in epidermal cells, one can conclude that epinephrine synthesis is of major significance in the epidermis.

Scheme I presents the biosynthetic pathway for epinephrine from L-tyrosine showing the important control points in the biosynthesis of this hormone. The availability of the essential coenzyme/electron donor tetrahydrobiopterin for TH, and the degradation of norepinephrine by MAO and COMT represent key points of regulation.

Since keratinocytes express a high density of beta-2-adrenoceptors, which appear to be directly involved in calcium homeostasis (7, 8), it seems plausible that the production of epinephrine in keratinocytes of the human epidermis is of major importance in this signal transduction system. The data presented herein support the regulation of epinephrine biosynthesis by high intracellular calcium via inhibition of NADPH-dependent reduction of biopterin to tetrahydrobiopterin. Further support for such a feedback loop by intracellular calcium comes from the observation that keratinocytes established in culture medium containing high calcium concentrations (1.5×10^{-3} M) show a 30-40% decrease in the expression of beta-2-adrenoceptors compared to cells grown in the same medium with low calcium concentrations (0.1×10^{-3} M) (5).

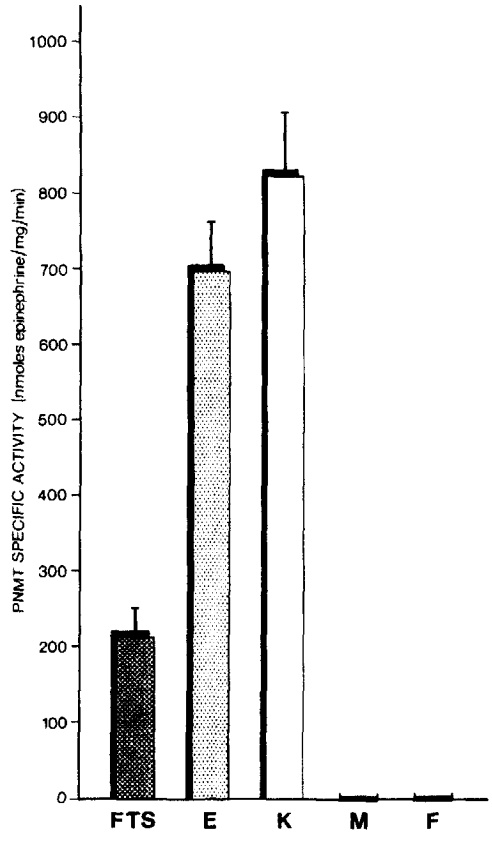


Figure 3.
Duplicate assays for PNMT activities were performed on cell extracts from full thickness skin (FTS), epidermal suction blister roofs (E), human keratinocytes (K), human melanocytes (M) and human fibroblasts (F). Reactions contained 100 µl of cell extract, 100 µl of tris-HCl buffer (0.05 M) pH 7.5 containing 5.0 µmoles of norepinephrine and 10 µl of ^{3}H methyl-labelled S-adenosyl-L-methionine (SAM) (1.06 Ci/mM) 3.0 µmoles. Reactions were incubated at 25°C and stopped by the addition of 5% trichloroacetic acid. Specific activities were determined from reactions rates as nmoles epinephrine formed/mg protein/minute.

The question remains, why do melanocytes in the epidermis, and fibroblasts in the dermis, express beta-2-adrenoceptors without showing any catecholamine biosynthetic capability? In the epidermis, it seems plausible that keratinocyte-derived epinephrine regulates beta-2-adrenoceptor expression on melanocytes. However, in the dermal compartment, epinephrine could originate via the vascular supply or from sympathetic nerve endings. Recently, it has been shown that beta-2-adrenoceptors are only expressed on pigmented primary and metastatic melanotic melanoma cells, whereas in amelanotic (non-pigmented) clones (n = 3), this signal transduction system is absent (6). These results suggest that epinephrine biosynthesis in human keratinocytes not only regulates calcium homeostasis in the epidermis but may be of importance in the regulation of melanin biosynthesis itself (24, 25). The recognition that epinephrine is produced directly in the human

epidermis by keratinocytes will certainly offer new insights into the pathophysiology in a number of skin disorders.

REFERENCES

1. Duell, E.A. (1980) *Biochem. Pharmacol.* **29,** 97-101.
2. Gazith, J., Cavey, M.T., and Cavey, D. (1983) *Biochem. Pharmacol.* **32,** 397-403.
3. Bristow, M.R., Ray, E.H., and Port, J.D. (1990) *Circulation (Suppl.)* **82,** 12-25.
4. Steinkraus, V., Steinfarth, M., Körner, C., and Mensing, H. (1992) *J. Invest. Dermatol.* **98:4**; 475-480.
5. Schallreuter, K.U., Pittelkow, M.R., Swanson, N.M., and Steinkraus, V. (1992) *Arch. Derm. Res.* (in press).
6. Schallreuter, K.U., Wood, J.M., Ehrke, C., and Lemke, R. (1992) *Biochim. Biophys Acta* (in press).
7. Koizumi, H., Zasui, L., Fukaya, T., Ohkawara, A., and Ueda, T. (1991) *J. Invest. Dermatol.* **96,** 234-237.
8. Steinkraus, V., Nose, M., Mensing, H., and Körner, C. (1990) *Br. J. Dermatol.* **123**, 163-170.
9. Boyce, S.T. and Harm, R.G. (1983) *J. Invest. Dermatol.* **81,** 335-405.
10. Hennings, H. and Holbrook, K.A. (1983) *Exp. Cell Res.* **143,** 127-142.
11. Durham, A.C. and Walton, J.M. (1982) *Biosci. Rep.* **2,** 15-30.
12. Watt, F.M., Mattey, D.C., and Garrod, D.R. (1984) *J. Cell Biol.* **99,** 2211-2215.
13. Eedy, D.J., Canavan, J.P., Shaw, C., and Trimble, E.R. (1990) *Br. J. Dermatol.* **122,** 477-483.
14. Hunt, G., Cresswell, J.E., Donatien, P.D., and Thody, A.J. (1992) *J. Invest. Dermatol.* **98:4**, 508.
15. Cassatella, M.A., Anegon, I., Cuturi, M.C., Griskey, P., Trinchieri, G., and Perussia, B. (1989) *J. Exp. Med.* **169,** 549-567.
16. Beutler, B. (1992) *Tumor Necrosis Factors*, Raven Press, NY.
17. Stryer, L. (1988) Biochemistry IIIrd Edition, W.H. Freeman and Co., NY **1025**.
18. Elayan, H., Kennedy, B., and Ziegler, M.G. (1990) *Archiv. Dermatol. Res.* **282,** 194-197.
19. Johansson, O., Olsson, A., Enhamore, A., Hammar, H., and Goldstein, M. (1987) *Acta. Derm. Venereol.* (Stockh) **67,** 1-7 (1987).
20. Nichol, C.A., Smith, G.K., and Duth, D.S. (1985) *Ann. Rev. Biochem.* **54**, 729-764.
21. Hogenkamp, H.P.C., Ghambeer, R.K., Brownson, C., Blakley, R.L., and Vitols, E. (1968) *J. Biol. Chem.* **243,** 799-808.
22. LePoole, I.C., Vanden Wijngaard, R.M.J.G.J., Smit, N.P.M., Westerhof, W., and Pavel, S. (1991) *IIIrd Proc. Eur. Soc. Pigment Cell Res.* **66**.
23. Ito, S., Jimbow, K., Kasuga, T., and Ishikawa, K. (1987) *Biochem. Pharmacol.* 36, 2007-2011.
24. Schallreuter, K.U. and Wood, J.M. (1989) *Biochim. Biophys. Acta* **997**, 242-247.
25. Wood, J.M. and Schallreuter, K.U. (1991) *Biochim. Biophys. Acta* **1074**, 375-385.