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MELITTIN-STIMULATED ARACHIDONIC ACID METABOLISM BY CULTURED MALIGNANT HUMAN EPIDERMAL KERATINOCYTES

Robert H. Rice and Lawrence Levine*

Charles A. Dana Laboratory of Toxicology, Harvard School of Public Health
Boston, Massachusetts 02115
*Department of Biochemistry, Brandeis University,
Waltham, Massachusetts 02254

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Upon melittin stimulation, cultured SCC-13 keratinocytes release prostaglandins E_2, $F_{2\alpha}$, 6-keto-$F_{1\alpha}$, thromboxane B_2, leukotriene B_4, and 6-sulfido-peptide-containing leukotrienes (SRS) into serum free medium. Release of prostaglandins E_2, $F_{2\alpha}$, and SRS, normalized to cell protein, is 3- to 10-fold higher from rapidly growing than confluent cultures. Cells growing with hydrocortisone in the medium produce approximately twice the level of the cyclooxygenase-mediated metabolites PGE_2 and $PGF_{2\alpha}$ as those without hydrocortisone, but similar levels of the lipoxygenase-mediated metabolite SRS. The results demonstrate the potential utility of squamous carcinoma lines for investigating biochemical pathways of arachidonic acid metabolism in keratinocytes.

Recent progress in serial cultivation of keratinocytes from a variety of species including human (1), mouse (2) and rat (3) provides much opportunity to investigate differentiated function and physiological response in this cell type (4). For example, in the SCC-13 keratinocyte line, derived from a squamous cell carcinoma of human epidermis (5), expression of individual differentiation markers can be modulated substantially by corticosteroids, retinoids and growth arrest (6,7) and environmental compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (8). Since much remains to be learned of the biochemical mechanisms by which physiological and toxic agents activate keratinocytes to produce arachidonic acid metabolites, including modulation among the various possible pathways, the present research explores the usefulness of SCC-13 cells for studying such phenomena.

Abbreviations: SRS, 6-sulfidopeptide-containing leukotrienes; LTB_4, leukotriene B_4; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TxB_2, thromboxane B_2; 6-keto-$PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$.

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MATERIALS AND METHODS

SCC-13 keratinocytes were grown in Dulbecco-Vogt Eagle's medium supplemented with 5°/° fetal bovine serum and hydrocortisone (0.4 μg/ml) as specified. In initial experiments, the cells were grown with a feeder layer of lethally irradiated mouse 3T3 (1). For adequate growth in later experiments without feeder support, the cultures were passaged at densities at least approximately 5 x 10^5 cells per 100 mm dish.

To measure arachidonic acid metabolites, cultures were rinsed vigorously once with isotonic 0.5 mM EDTA (9) if feeder layer support was employed, twice with serum free medium and held 15 min in the culture incubator with 2 ml (60 mm dish) or 6 ml (100 mm dish) of serum free medium. Melittin was then added in 2 to 40 μl of phosphate buffered saline and the incubation continued for the times indicated. The decanted medium was frozen quickly on dry ice and stored frozen until assay. PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TxB_2, LTB_4 and SRS were analyzed by radioimmunoassay. Most of these assays were performed with serologically homogeneous antisera, that of SRS was not. LTC_4, LTD_4, 11-trans-LTD_4, and LTE_4 reacted 100, 43, 48, and 6°/°, respectively, with this antiserum (10) and the data were calculated from the inhibition of LTD_4. Radioimmunoassays for all arachidonic acid metabolites were performed in 3.5 ml polypropylene test tubes. The diluent for all reagents was 0.01 M Tris-HCl (pH 7.4) containing 0.014 M NaCl and 0.1°/° gelatin.

RESULTS

In initial experiments, conditioned serum free medium from SCC-13 cultures contained several easily detectable immunologically active arachidonic acid metabolites (PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TxB_2, LTB_4, SRS) after stimulation by melittin. The low levels of PGE_2 and $PGF_{2\alpha}$ found in this medium were increased 3 to 8 fold by 20 min treatment with melittin at 10 μg/ml. As generally found in such treatment of other cell types (11), the stimulation occurred quickly. Near maximal accumulation of PGE_2 and $PGF_{2\alpha}$ occurred in 10 to 20 min. In a preliminary experiment, the calcium ionophore A 23187 (2 to 200 μM) was considerably less effective in stimulating arachidonic acid metabolism than melittin.

During these experiments, the 3T3 feeder cells were found capable of substantial production of such compounds. With PGE_2 and $PGF_{2\alpha}$, the keratinocyte contribution was clearly evident after removal of residual 3T3 using isotonic EDTA (9). In the case of 6-keto-$PGF_{1\alpha}$, however, 3T3 alone at feeder layer density (one-third confluence) produced an order of magnitude higher amounts than the keratinocyte cultures grown to confluence with feeder layer support. When SCC-13 cells were grown in the absence of 3T3, only low levels of 6-keto-$PGF_{1\alpha}$, LTB_4 and TxB_2 were detected upon melittin

treatment. To eliminate the potential contribution of any remaining 3T3 in later experiments, SCC-13 cells were passaged at high density without feeder support.

In three subsequent experiments, SCC-13 cells produced higher levels of those metabolites measured (PGE_2, $PGF_{2\alpha}$, SRS) during rapid growth, especially when the medium contained 1 μM hydrocortisone, than at confluence. Results of a representative experiment are shown in Figure 1, where the values reported have been normalized to total cell protein per culture. For each of these 3 metabolites measured, 3- to 4-fold higher levels were produced by the cultures still in log phase of growth (3 days) than those in which the individual colonies had merged at confluence (6 days). Twofold higher levels of PGE_2 and $PGF_{2\alpha}$ were produced by cultures grown in the presence of hydrocortisone during log phase. By contrast, retinoic acid in the medium (0.1 μg/ml) had little effect on the metabolite production by these cells.

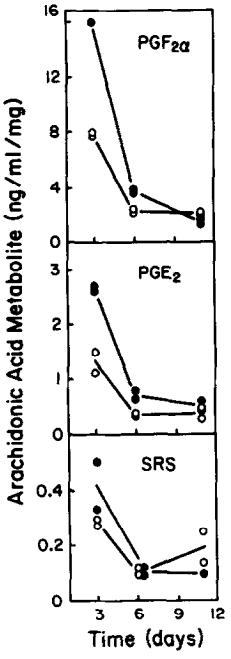

Fig. 1 Production of PGE_2, $PGF_{2\alpha}$ and SRS in 100 mm cultures grown without 3T3 support and with (●——●) or without (o——o) added hydrocortisone. Confluence occurred approximately by day 6. Parallel cultures in duplicate were rinsed with serum free medium and treated with melittin (10 μg/ml) for 15 min. Graphed values were normalized to protein content (14) per culture, approximately 2.9, 11.4 and 12.4 mg (+ hydrocortisone) or 3.0, 7.6 and 7.6 mg (- hydrocortisone) on days 3, 6 and 11, respectively.

DISCUSSION

The results demonstrate that SCC-13 cells are capable of making arachidonic acid metabolites by both the cyclooxygenase and lipoxygenase pathways. The marked positive effect of hydrocortisone, which could reflect increased phospholipase or cyclooxygenase activity, is indicative of responsiveness of the growing cells to corticosteroids (8) despite the relative lack of keratinocyte character expressed until the cultures reach confluence (6). That the metabolic capability is considerably lower at confluence shows it is regulated differently from the biochemical differentiation markers studied to date. Increased levels of PGE_2 have been found in conditioned media of Friend erythroleukemia cells stimulated to differentiate by dimethylsulfoxide (12). Similarly, dimethylsulfoxide induced differentiation of the human promyelocytic cell line HL 60 was accompanied by increased phospholipase and cyclooxygenase activities (13).

Production of arachidonic acid metabolites may be an important aspect of pathogenic behavior of afflicted keratinocytes in prevalent diseases (e.g., psoriasis) and tumors of epidermis and related epithelia. The production of SRS and LTB_4, likely mediators of vascular and inflammatory events in skin, suggests such cultivated cells may prove useful in exploring the regulation of such metabolites and modulation by therapeutic agents. The conspicuous variability among tumors raises the possibility that other available keratinocyte carcinoma lines (5) may display physiologically important, perhaps novel properties in this regard. Indeed, since keratinocytes are now serially propagated in routine fashion even from normal epithelia and display intrinsic differences in culture (3), their repertoire of arachidonic acid metabolism appears open to investigation.

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