Membrane-bound Phospholipase C Activity in Normal and Psoriatic Epidermis

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We report the quantification of a membrane-bound phospholipase C in human epidermis which is active against the physiologically relevant substrate, phosphatidylinositol 4,5-bisphosphate. The level of this enzyme is significantly increased in the psoriatic lesion, both on a weight and protein basis. Etiological implications of this observation are discussed. ***Key words: Polyphosphoinositides; Epidermis; Psoriasis.***

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The phosphoinositol (PI) cycle is found in many mammalian cells. An extracellular 'first messenger' is recognized by an appropriate membrane receptor which, via a G protein, activates a membrane-bound polyphosphoinositide-specific phospholipase C (PLC). This enzyme cleaves phosphatidylinositol 4,5-

Table I. *Activity of membrane-bound phospholipase C in epidermis*

N=normal; PU=psoriasis uninvolved; PL=psoriasis lesion

Specimen No.	pmol/min/mg weight			pmol/min/µg protein		
	N	PU	PL	N	PU	PL
1	20.3	29.0	47.6	1.02	1.44	1.38
2	67.0	71.0	85.6	3.31	3.53	2.48
3	22.6	14.7	111.5	1.12	0.73	3.23
4	34.5	10.5	66.9	1.70	0.52	1.94
5	13.2	47.2	57.6	0.55	3.80	1.75
6	12.4	44.3	114.0	0.52	1.93	3.80
7	15.3	15.5	72.5	0.85	0.70	2.07
8	17.3	14.1	54.6	1.15	0.61	1.37
Mean	25.3	30.8	76.3	1.28	1.66	2.25
SD	18.2	21.6	25.3	0.90	1.33	0.87

bisphosphate (PIP_2) to yield two 'second messengers', diacylglycerol and inositol trisphosphate. The former activates protein kinase C, whilst the latter initiates a dramatic increase in cytosolic calcium levels. Many comprehensive reviews of the PI cycle are now available (1–4).

In some tissues activation of the PI cycle results in proliferation, via activation of a Na^+–H^+ antiport and consequent elevation of cytosolic pH (5). Another potential target for phosphorylation is lipomodulin, the endogenous inhibitor of phospholipase A_2 (6); this is one of the mechanisms leading to release of arachidonic acid and inflammatory eicosanoids. Since the skin disease psoriasis is characterized by chronic hyperproliferation and a dramatic increase in the production of eicosanoids, the PI cycle is a logical candidate in the search for the underlying biochemical abnormality (7). Here we present the first unequivocal demonstration of a membrane-bound PLC in human epidermis which can hydrolyse PIP_2, and report its levels in normal and psoriatic subjects.

MATERIALS AND METHODS

Subjects

Psoriatic patients were selected who had stable, chronic lesions which had not been treated for at least two weeks; biopsies were taken either from the central region of a well-established plaque or from the clinically healthy skin at least 20 cm distance from a lesion. Control specimens were obtained from the upper backs of paid volunteers with no personal or family history of psoriasis.

Samples of about 1 cm^2 were cut with a Castroviejo keratotome (set to 0.3 mm) after cooling the skin with ethyl chloride spray, and were either processed immediately or stored at −20°C prior to use. Direct histological examination confirmed that essentially all epidermal tissue was included in the biopsies.

Phospholipase C assay

This was as described previously (8). In brief, all samples were weighed and homogenized in a buffer containing 20 mM tris, 1 mM $CaCl_2$ and 100 mM KCl (pH 7.4) using an all-glass Potter-type grinder. The homogenate was centrifuged (30 min, 40 000 g), the pellet washed by centrifugation and resuspended in buffer at 10 mg/ml to yield a crude membrane suspension. The substrate was prepared by sonification of 50 nmol PIP_2 (Sigma, St. Louis, MO), 0.5 µCi [^3H]-PIP_2 (NEN, Doorn, NL) and 0.4 mg cetrimide in 1 ml of the same buffer.

The reaction was initiated by the addition of 10 µl of the substrate mixture to 10 µl of the crude membrane preparation. After incubation for 10 min at 37°C, the reaction was stopped by the addition of 500 µl 1 N HCl and 500 µl chloroform/methanol (1 : 1). The mixture was agitated vigorously, the phases separated by a brief centrifugation and 500 µl of the upper (aqueous) phase removed for scintillation counting. All samples were measured in duplicate, and appropriate reaction-blanks were included with each batch of assays. After correction for the blank, activity was calculated as pmol PIP_2 hydrolysed per min.

Protein measurement

The protein content of the supernatant fraction of the epidermal homogenate was determined by direct fluorimetry (λ ex = 278 nm, λ em = 340 nm) with reference to a bovine serum albumin standard.

Statistical methods

A non-parametric test of significance (the Wilcoxon rank Order test for unpaired data) was used because of the skewed distributions of the data. All calculations were carried out using the SAS package and a VAX computer system.

RESULTS

In preliminary experiments the reaction was shown to be linear with respect to time and tissue concentration until about 25% of the substrate was utilised. Thus, using a suspension equivalent to 10 mg/ml fresh weight of epidermis, a reaction time of 10 min gave adequate sensitivity without exceeding the linear region of the kinetics.

Absolute values of PLC activity in healthy and psoriatic epidermis are shown in Table I. It is seen that the levels in psoriatic lesion are raised with respect to healthy epidermis, regardless of the reference variable employed (fresh weight, $p<0.005$; protein, $p<0.02$). By contrast, clinically uninvolved psoriatic biopsies were not significantly different to normal ($p<0.75$ for both reference variables).

DISCUSSION

It has been established that phorbol myristic acetate (an activator of protein kinase C) leads to the recruitment of G_0 cells in mouse epidermis, and that the proliferative response to injury can be blocked by amiloride, an inhibitor of the Na^+–H^+ pump (9). More recently, the incorporation of labelled inositol into inositol phospholipids and the release of inositol triphosphate from the phospholipid pool has been described using cultured murine keratinocytes (10). Our present observation that a crude membrane fraction from human epidermis can release water-soluble inositol derivatives from exogenous PIP_2 thus provides further evidence for the existence of the PI cycle in this tissue.

The increased membrane-bound PLC activity in psoriatic lesional epidermis is in line with an earlier publication showing a similar elevation of a cytosolic PLC which hydrolyses phosphatidylinositol (11). Although these data seem compatible with the concept of an imbalance in the PI cycle (7), several major limitations must be kept in mind. First, if the PI cycle is responsible for the transduction of proliferation-associated signals, it is likely to be expressed mainly or exclusively in cells of the germinative population. Since these are 7-fold more numerous in the psoriatic lesion (12), the PLC level per germinative keratinocyte could be normal or even reduced. Secondly, it must be emphasized that measurements of this kind tell us only the maximum potential catalytic activity of the enzyme, since PLC becomes independent of receptor or G protein stimulation at high (1 mM) calcium levels. Thus physiologically-relevant aspects of signal transduction such as negative feedback control (4), which operate via the G protein, remain unexplored.

More detailed studies of the various components of the epidermal PI cycle are necessary before any claim of an intrinsic psoriatic 'abnormality' can be established. Further work in this direction is in progress in our laboratories.

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