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Cyclic GMP Metabolism in Psoriasis: Activation of Soluble Epidermal Guanylate Cyclase by Arachidonic Acid and 12-Hydroxy-5,8,10,14-Eicosatetraenoic Acid

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Soluble guanylate cyclase activity was measured in normal and psoriatic human epidermis. The specific activity of guanylate cyclase was determined to be increased 10-fold and 3-fold in involved and uninvolved epidermis of psoriatics, respectively, compared to normal epidermis. Arachidonic acid (5 to 100 μM) or 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE) (5 to 50 μM) stimulated guanylate cyclase activity from involved epidermis 2- to 3-fold and from uninvolved epidermis up to 2-fold, but these fatty acids had no effect on the activity of this cyclase from normal epidermis. These results indicate that there is an increase in the cGMP biosynthetic capacity of involved epidermis from psoriatics that derives from a markedly increased specific activity of guanylate cyclase and an alteration in a property of this enzyme activity which renders it responsive to fatty acids reported to accumulate in this lesion. These observations are consistent with the report that an elevated steady-state level of cGMP is one of the consequences of the strikingly altered metabolism of cGMP in psoriatic epidermis.

Cellular accumulation of cyclic 3′,5′ guanosine monophosphate (cGMP) has been shown to occur in association with the action of numerous hormonal agents and a variety of other naturally occurring, biologically active substances including mitogens [1]. Psoriasis is a nonmalignant proliferative skin condition in which cellular cGMP steady-state levels have been shown to be increased [2,3]. The biochemical basis for the increased levels of cellular cGMP that occur in psoriasis or in association with other inducible alterations in cell function has not yet been defined. Membrane-active agents which promote cellular cGMP accumulation have not been shown to affect the activities of either guanylate cyclase or of cGMP phosphodiesterase after cell disruption [1]. This has led to the suggestion [4] that enzymes involved in the metabolism of cGMP may be regulated indirectly with respect to these biological signals by substances generated within the cell upon stimulation of membrane receptors.

Polyunsaturated fatty acids were among the first defined naturally occurring substances shown to activate guanylate cyclase [5–10]. The soluble form of guanylate cyclase from human platelets was shown to be stimulated by arachidonic acid and structurally related fatty acids as a result of a noncooperative interaction with an enzyme site(s) exhibiting relatively high affinity (Ka = 2.1 μM) for the lipid component [4].

Hammarstrom et al [11] have reported that involved psoriatic epidermis has strikingly elevated concentrations of arachidonic acid (i.e., 25-fold elevated) and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE) (i.e., 81-fold elevated). This disclosure combined with the discovery that a soluble guanylate cyclase from at least one source is stimulable by a specific class of polyunsaturated fatty acids raised a question regarding the influence that the fatty acids which accumulate in psoriasis may have on cGMP metabolism in epidermis affected by this disorder.

In this communication, the activity of guanylate cyclase in normal and psoriatic human epidermis has been investigated, as well as the effect of arachidonic acid and HETE on this enzyme activity. The results indicate that soluble guanylate cyclase activity is markedly increased in lesional epidermis, and that the enzyme from this site can be stimulated by micromolar concentrations of arachidonic acid and HETE, whereas the activity from normal epidermis cannot.

MATERIALS AND METHODS

Materials

GTP, dithiothreitol (DTT), and bovine serum albumin were obtained from Sigma. Arachidonic acid was purchased from Nu Chek Prep. All other reagents were from commercial sources and of the highest purity available. Antibodies against cGMP were prepared in this laboratory from goats. Succinyl-cGMP-tyrosine methyl ester from Sigma was iodinated according to the method of Steiner, Parker, and Kipnis [12]. HETE was generated from arachidonic acid by incubation with purified cow platelet lipoxygenase and purified as previously described [13].

Preparation of Soluble Guanylate Cyclase

Human skin slices were obtained without anesthesia from 7 normal volunteers and 4 untreated psoriatic patients. Informed consent was obtained from all subjects. Both uninvolved and involved areas of skin were sampled from psoriatic patients; the uninvolved specimen was taken from an area of skin at least 5 cm from a well-developed plaque. A Castroviejo keratotome set to a tissue depth of 0.1 to 0.2 mm for normal and uninvolved epidermis and 0.4 mm for involved epidermis produced slices 3 cm wide by 8 cm long, which were immediately frozen in liquid nitrogen. Histological examination revealed that the samples were predominantly epidermis with little dermis, but the degree of dermal contamination was variable. The frozen samples were pulverized by mortar and pestle in liquid nitrogen and homogenized with a Brinkman Polytron in 50 mM Tris-HCl, pH 7.5, containing 2 mM DTT. The homogenates were centrifuged for 60 min at 105,000 ×*g* to separate the soluble and particulate cellular fractions. The soluble fraction was removed and assayed within 30 min.

Guanylate Cyclase Assay

Activity of the enzyme was measured by a previously described modification [14] of the method of Kimura and Murad [15]. Final concentrations of the components in the cyclase assay (80 μl) were 1 mM GTP, 3 mM $MnCl_2$, 5 mM 3-isobutyl-l-methylxanthine and 2 mM DTT in 50 mM Tris-HCl, pH 7.5. Fatty acids tested as possible effectors of guanylate cyclase were added prior to addition of the reaction mixture and enzyme to reaction tubes as previously described [13].

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Abbreviations:
cGMP: cyclic 3′,5′ guanosine monophosphate
DTT: dithiothreitol
HETE: 12-hydroxy-5,8,10,14-eicosatetraenoic acid

Solvent, containing the lipid component, was evaporated under vacuum (10s to 20s) before addition of the enzyme extract (40 μl containing 0.12 to 1.0 μg of protein) followed by the reaction mixture (40 μl) 5 sec later. Appropriate controls representing solvent alone were included [13]. The cyclase reaction which was determined to be linear with respect to time for at least 30 min was conducted at 37°C for the times designated and terminated by the addition of 80 μl of 110 mM sodium acetate, pH 4.0, containing 11 mM EDTA followed by heating at 90°C for 3 min. Blank reactions were represented by enzyme extract added to the sodium acetate/EDTA solution heated at 90°C for 3 min followed by the addition of the reaction mixture. The cGMP formed was converted to the 2′-O-acetyl derivative and assayed by radioimmunoassay as previously described [13,14]. The protein concentration of the extract was determined by the method of Bradford [16].

Statistical Analysis

All determinations were conducted in triplicate. Specific activities are expressed as picomoles/min/mg protein ± standard error of the mean. Significance levels were established by a paired, 2-sided Student *t*-test.

RESULTS

Guanylate Cyclase Activity

The study of guanylate cyclase from human epidermis was confined to the soluble fraction (105,000 *g*) of activity obtained after disruption of rapidly frozen tissue. Guanylate cyclase activity from tissue representing each of the clinical states (i.e., normal, uninvolved, and involved) was determined to be linear for at least 30 min (Fig 1). Magnesium (5 mM) was determined to support only 10 to 15% and Ca^{2+} (5 mM) less than 10% of the activity obtained with Mn^{2+} (not shown). It was noted in the initial studies, such as the one depicted in Fig 1, that the specific activity of the guanylate cyclase from involved epidermis was considerably greater than that from normal or uninvolved tissue samples; the specific activity of the uninvolved was intermediate with respect to normal and involved epidermis.

A more thorough examination of guanylate cyclase activity from these 3 different sources showed the specific activity to be 10-fold greater in involved compared to normal epidermis (Table I). The specific activity of the cyclase from uninvolved epidermis was significantly greater (ca. 3-fold) than the activity from normal tissue, but only one-third of the activity measurable in extracts from involved tissue.

These marked differences in guanylate cyclase activities from the different tissue sources were examined kinetically (Fig 2) and found to be due clearly to an increased maximum enzyme velocity. The value of Km for GTP in the presence of saturating

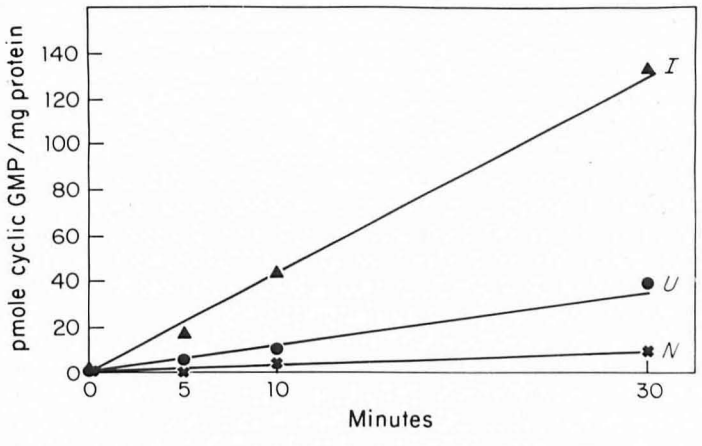

FIG 1. Time course of soluble epidermal guanylate cyclase activity. Reactions were conducted as described in the Methods section in the standard reaction mixture. Enzyme activity was obtained from χ, normal epidermis (N); ●, uninvolved (U); and ▲, involved (I) psoriatic epidermis.

TABLE I. *Guanylate cyclase specific activities*

	Normal (N)	Uninvolved (U)	Involved (I)
	pmoles cGMP/min/mg protein		
	0.40	1.55	4.41
	0.51	1.81	3.69
	0.47	0.48	1.98
	0.24	0.58	3.55
	0.23		
	0.17		
	0.37		
Mean ±SEM	0.34 ± 0.06	1.11 ± 0.34	3.41 ± 0.51
		U/N 3.26 ($p < .05$)	I/N 10.03 ($p < .001$)
			I/U 3.07 ($p < .01$)

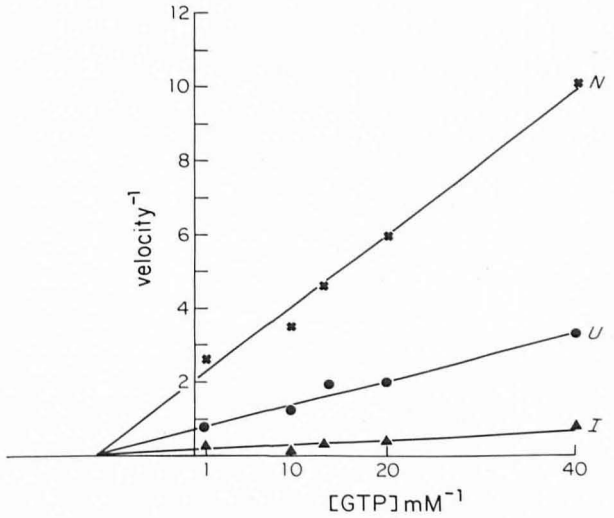

FIG 2. Double reciprocal plot of guanylate cyclase activity from 3 types of epidermis as a function of GTP concentration. Reactions were carried out as described in Methods for 10 min in the presence of a constant concentration of Mn^{2+} (3 mM) and a variable concentration of GTP 25 μM to 1 mM). Velocity^{-1} represents (pmoles cGMP/min/mg protein)$^{-1}$. Source of enzyme activity: χ, normal epidermis (N); ●, uninvolved (U) and ▲, involved (I) psoriatic epidermis.

Mn^{2+} (3 mM) is approximately 100 μM for the enzyme activities from these 3 different types of epidermis.

Effect of HETE and Arachidonic Acid on Enzyme Activity

HETE and arachidonic acid, which have been shown to be markedly elevated in psoriatic lesions [11], have virtually no influence, in a concentration range of 5 to 50 or 100 μM (i.e., arachidonate), on soluble guanylate cyclase from normal human epidermis (Table II). A markedly different sensitivity to these fatty acids is exhibited by the cyclase from involved epidermis; HETE or arachidonic acid at the lowest concentration of the fatty acid tested (i.e., 5 μM) promoted significant activation of the already 10-fold enhanced basal cyclase activity from involved epidermis. Maximum activation was about 3-fold with 50 μM HETE or 100 μM arachidonate. Fig 3 provides a graphic representation of the concentration dependence of HETE activation. With Mg^{2+} instead of Mn^{2+} as the divalent cation used to support activity, arachidonic acid (25 μM) produced an activation of 4-fold (not shown). A 1.5- to 2-fold activation of the guanylate cyclase from uninvolved epidermis was also observed with arachidonic acid or its 12-hydroxy analogue, but the degree of variation encountered with the enzyme from this group of tissues yielded significant results with only the highest or next to highest (i.e., HETE) concentration of the lipid components tested. It is apparent, however, from these results that the

TABLE II. *Effect of HETE or arachidonic acid on guanylate cyclase activity*

Origin of enzyme activity	Percent of basal activity from corresponding tissue[a]					
	μM HETE			μM Arachidonate		
	5	25	50	5	25	100
Normal (N)	103 ± 10	89.7 ± 4.1	107 ± 14	88.5 ± 6.3	106 ± 11	104 ± 14
Uninvolved (U)	129 ± 12	150 ± 13[d]	171 ± 26[b]	190 ± 56	212 ± 66	234 ± 65
Involved (I)	188 ± 38[b]	236 ± 16[c]	288 ± 30[d]	208 ± 22[d]	281 ± 33[d]	320 ± 39[d]

[a] Basal activities from normal, uninvolved, and involved tissues were 0.34, 1.11, and 3.41 pmoles/min/mg protein, respectively (see Table I). Activities expressed as mean ± SEM.
[b] $p < .05$.
[c] $p < .01$.
[d] $p < .001$.

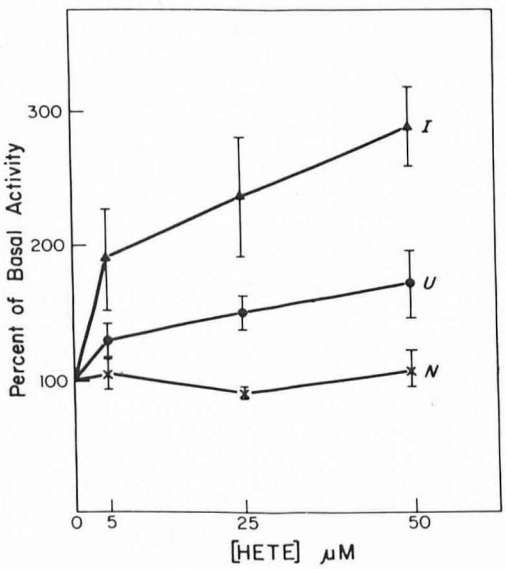

FIG 3. Percent basal guanylate cyclase specific activity as a function of HETE concentration. Reactions were conducted as described in the Methods section in the presence of a variable concentration of HETE. Source of enzyme activity: χ, normal epidermis (N); ●, uninvolved (*U*), and ▲ involved (*I*) psoriatic epidermis. *Points* represent means ± SEM.

enzyme from involved and uninvolved epidermis can be activated by HETE and arachidonic acid, whereas the cyclase from normal epidermis cannot.

DISCUSSION

This is the first report of the properties of the enzyme catalyzing the biosynthesis of cGMP in human epidermis and of the characteristics of the guanylate cyclases in normal and psoriatic epidermis that distinguish the activities in these 2 tissues.

The soluble guanylate cyclase from normal human epidermis exhibits certain characteristics common to the enzyme activity from other tissue sources including rat epidermis [17], which was also studied for comparison in this investigation. The preferred specificity for Mn^{2+} as the divalent metal compared to Mg^{2+} or Ca^{2+} and the abundance of activity in the soluble fraction of disrupted cells are characteristics of human and rat epidermal guanylate cyclase ordinarily found with this activity from other sources [1,18]. The soluble enzyme from rodent and human epidermis (i.e., normal, involved, and uninvolved) is not responsive, however, to activation by Triton X-100 (0.5%) (not shown) as are the soluble activities from other sources [1,18], with the exception of human platelets [4].

One characteristic of the species of soluble guanylate cyclase from involved psoriatic epidermis which clearly distinguishes it from the enzyme activity in normal human epidermis is a sensitivity to stimulation by arachidonic acid and HETE. Fatty acids have been shown to stimulate both soluble [4,6,19] and particulate forms [8–10] of guanylate cyclase from several sources, but concentrations of the fatty acids ordinarily reported to be effective are 1 to 2 orders of magnitude greater than those shown to be stimulatory for the cyclase from human psoriatic epidermis. Significant activation by concentrations of fatty acids below 10 μM has been reported previously only for the soluble enzyme from human platelets [4]; the platelet enzyme exhibits a Ka value for arachidonic acid of 2 μM and an interaction of one mole of polyunsaturated fatty acid per catalytic site of enzyme. The ineffectiveness of Triton X-100 and effectiveness of 5 μM arachidonic acid or HETE to produce approximately half-maximal activation of the cyclase from human psoriatic epidermis suggests that a similar specificity with regard to activation by fatty acids may exist for soluble guanylate cyclases from psoriatic epidermis and human platelets. Fatty acid specificity was not determined in the present studies, but the lipid components tested (i.e., arachidonic acid and HETE) could be considered to be of special importance because of their marked accumulation compared to other fatty acid metabolites examined in the psoriatic lesion [11].

From the data reported by Hammarstrom et al [11], free arachidonic acid and HETE can be calculated to accumulate in involved epidermis to levels equivalent to approximately 120 and 13 μM, respectively. These concentrations are within the range in which both fatty acids are effective in activating psoriatic epidermal guanylate cyclase. On the other hand, the concentration of arachidonate and HETE reported by these investigators to be present in uninvolved epidermis would produce little or no effect.

To minimize any influence that endogenous fatty acids in the tissue samples analyzed (e.g., especially from involved epidermis) may have had on the activity of guanylate cyclase measured *in vitro*, tissues were diluted 50- to 100-fold in the cyclase reaction. Concentrations of arachidonic acid and HETE as great as 2.4 and 0.26 μM, respectively, could conceivably have been present in reaction mixtures containing extracts from psoriatic epidermis. It is possible, therefore, that the markedly increased guanylate cyclase activity measured in extracts from psoriatic epidermis may be partially attributable to an influence of endogenous arachidonic acid. However, the apparently greater sensitivity to exogenous arachidonic acid of guanylate cyclase from involved compared to uninvolved psoriatic epidermis and the fact that additional (2- and 3-fold) dilutions of the extracts from psoriatic tissue were not found to alter the specific activity would argue against this possibility.

The major problem inherent to studies of biopsied psoriatic epidermis is the potential interference deriving from cells other than those of epidermal origin. In the case of the changes seen in guanylate cyclase activity and the alteration in sensitivity to stimulation by arachidonic acid and HETE in involved and uninvolved epidermis, the major source of error would be expected to derive from contaminating platelets. These blood elements exhibit approximately 100 times the soluble guanylate cyclase specific activity found in normal epidermis and a sen-

sitivity to similar concentrations of arachidonic acid (and HETE, unpublished observation), but less than half of the responsiveness [4] exhibited by the cyclase from involved epidermis. It is, however, highly unlikely that the 10-fold increase in the specific activity of guanylate cyclase of psoriatic epidermis can be accounted for by contaminating platelets. It would require a volume of whole blood 25 times greater than the mass of tissue analyzed to account for the 10-fold increase in guanylate cyclase activity by contaminating platelets, since they represent only 0.4% of the total volume of whole blood.

In the present study involved and uninvolved epidermis of psoriatic patients was compared with epidermis from unaffected volunteers. This comparison revealed that the uninvolved epidermis exhibits characteristics of guanylate cyclase that are qualitatively similar to, but quantitatively of a lesser magnitude than, the alterations that characterize the cyclase from lesional epidermis. This apparently altered state of guanylate cyclase in uninvolved epidermis suggests a predisposition for the lesion and "pre-lesional" changes in other components that may underly the disease process. The apparently altered sensitivity of guanylate cyclase activity from psoriatics to fatty acids suggests a genetically-linked alteration in primary enzyme structure, or the production of a component which renders this enzyme susceptible to fatty acid activation.

Although existing knowledge is incomplete concerning all of the factors that contribute to maintaining cellular cGMP concentration, the results of the present study indicate that the steady-state level of cGMP in psoriatic tissue should be considerably different from that of normal or uninvolved epidermis. The 10-fold greater specific activity of guanylate cyclase and 3-fold stimulatory effect of the elevated endogenous arachidonic acid and/or HETE in lesional epidermis would provide for a 30-fold greater catalytic capacity to generate cGMP in the involved tissue. This enhanced biosynthetic capacity could provide for an increase in the steady-state level of cGMP which has been observed in psoriasis and postulated to be associated with enhanced rates of cell proliferation [1,20,21].

The importance of these findings to the pathophysiology of psoriasis cannot be defined at present, but the observations provide an explanation for the increase in cGMP levels observed in involved psoriatic epidermis and lend further support to the concept that specific fatty acids and their metabolites may represent modulators of cellular guanylate cyclase activity [4,5]. Studies are now in progress examining the role of cyclic GMP phosphodiesterase in the altered metabolism of this nucleotide in psoriasis.

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