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REPORTS

STUDIES IN PORPHYRIA

VI. Biosynthesis of Porphyrins in Mammalian Skin and in the Skin of Porphyric Patients

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Porphyrin biosynthesis in mammalian skin and in skin obtained from patients with selected types of porphyria has been studied. Cutaneous porphyrinogenesis required the precursor δ-aminolevulinic acid (ALA) which, when added to murine, rat, and human skin in vitro, was rapidly converted to porphyrins. Total porphyrin content was quantitated by fluorescence assay, and spectral studies indicated that more than 80% of the porphyrin produced was protoporphyrin. The majority of skin porphyrinogenesis occurred in epidermis or in epidermal derivatives such as hair roots. Known inducers of hepatic δ-aminolevulinic acid synthetase (ALAS), the rate-limiting enzyme for heme biosynthesis, were not inducers when added to skin in vitro.

Skin from patients with acute intermittent porphyria demonstrated a 43% decrease in cutaneous porphyrin production as compared to unaffected normals. This is consistent with the known deficiency of uroporphyrinogen synthetase that has been previously demonstrated in the liver and red blood cells of these patients. Porphyrinogenesis in skin of patients with porphyria cutanea tarda was not different from controls.

These studies demonstrate that skin has the enzymatic capacity to synthesize porphyrins from added ALA and that cutaneous porphyrinogenesis from ALA is deficient in patients with acute intermittent porphyria.

Porphyrins are biochemical intermediates in the synthesis of heme, a moiety that occurs in all living cells and is a basic requirement for aerobic and anaerobic metabolism. For example, heme is the prosthetic group for a number of different proteins including cytochromes, catalases, peroxidases, as well as hemoglobin.

Heme biosynthesis is initiated in the mitochondrion of the cell where, in the presence of the enzyme δ-aminolevulinic acid synthetase (ALAS), succinyl CoA and glycine are combined to form the aminoketone δ-aminoleuvulinic acid (ALA). Early studies by Granick and Urata [1] showed that the activity of this enzyme in liver is rate-limiting for heme synthesis, whereas the remaining enzymes in the pathway are usually present in sufficient nonlimiting amounts to convert any enzymatically formed ALA to porphyrins and/or heme (Fig. 1). Following the formation of ALA, additional enzymatic activity results in the production of porphobilinogen (PBG), a monopyrrole, and subsequently in the formation of porphyrinogens that are tetrapyrroles consisting of four monopyrroles linked by methene bridges. With the insertion of iron into the tetrapyrrole protoporphyrin IX, heme is produced. Porphyrins are oxidized porphyrinogens and it is the latter nonresonating compounds that are the true intermediates of the heme pathway in vivo (Fig. 1). Oxidized porphyrins are fluorescent. Their importance in clinical dermatology relates to the fact that in selected types of porphyria they may accumulate in the skin and, following exposure to certain wavelengths of light, result in cutaneous photosensitization [2–4]. These fluorescent chemicals absorb light intensely in the 400-nm range, the so-called Soret band, and by transferring this absorbed energy to cellular structures, photosensitizing damage can occur. Cutane-

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Abbreviations:
AIA: allylisopropylacetamide
AIP: acute intermittent porphyria
ALA: δ-aminolevulinic acid
ALAS: δ-aminolevulinic acid synthetase
PBG: porphobilinogen
PCT: porphyria cutanea tarda
URO I: uroporphyrinogen I
UROS: uroporphyrinogen synthetase

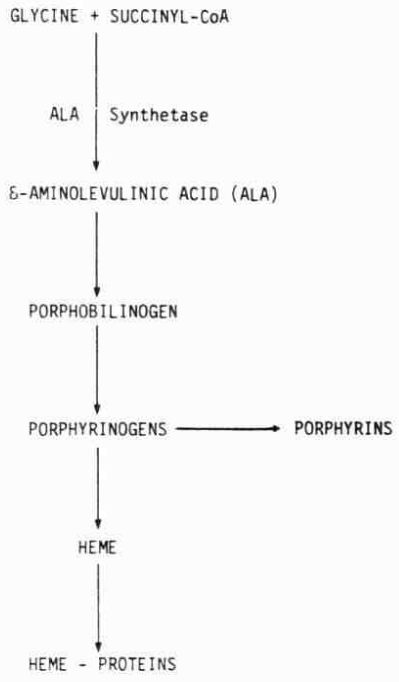

FIG. 1. Outline of the heme biosynthetic pathway.

ous photosensitivity may be associated with increased porphyrin levels in the skin, erythrocytes, and/or plasma. Furthermore, relatively simple diagnostic procedures can detect excessive porphyrins in the urine and feces of affected individuals [5,6].

The requirements for heme synthesis are greatest in the bone marrow and in the liver. Bone marrow heme is used for the production of hemoglobin in the erythrocyte, whereas hepatic heme is incorporated into a variety of proteins by the liver. Under normal circumstances the heme pathway is precisely controlled such that only trace amounts of the porphyrins are present in tissues such as bone marrow and liver.

Diseases reflecting derangements in the control of porphyrin–heme biosynthesis, known as the porphyrias, are of considerable medical interest since selected biochemical aberrations of heme synthesis can be correlated with certain clinical manifestations of these disorders.

Many different tissues are known to be capable of porphyrin synthesis from added ALA [7,8]. However, no studies have been carried out to evaluate porphyrin biosynthesis in the skin although Pathak and Burnett [9,10] did quantitate the porphyrins present in normal rat and human skin, in animals with drug-induced porphyria, and in patients with porphyria cutanea tarda. Recent studies by Bonkowsky et al [11] and by Sassa et al [12] have shown that by adding ALA or PBG to skin fibroblasts grown in tissue culture, it is possible to assess the activity of the enzyme uroporphyrinogen synthetase (UROS). This enzyme functions to convert PBG to uroporphyrinogen I. The activity of UROS has been shown to be deficient in both the liver and red blood cells of patients with acute intermittent porphyria (AIP) [13–15]. Affected individuals as well as latent carriers of this autosomal dominant defect demonstrate decreased activity of UROS. No previous studies have been carried out to assess the feasibility of using skin biopsies incubated in organ culture as a method with which to measure this enzyme activity in patients with AIP.

The present study was designed to assess several aspects of porphyrin-heme biosynthesis in the skin: (1) the capacity of mammalian skin to convert ALA to porphyrins using an organ culture system; (2) the ability of known inducers of hepatic heme synthesis to induce porphyrinogenesis in cutaneous tissue in vitro; (3) the feasibility of using skin biopsies from patients with AIP to demonstrate deficient UROS activity in cutaneous tissue.

MATERIALS AND METHODS

Porphyrinogenesis in skin. Skin biopsies (4–6 mm in diameter) were obtained from mature male Sprague-Dawley rats, from Swiss–Webster mice, from normal adult males undergoing hair transplantation, and from patients with AIP or porphyria cutanea tarda (PCT). The skin samples were finely minced with sterile scissors, incubated for 24 hr in standard tissue culture media using a modification of the liver cell culture technique described by Granick [16]. The major modification involved the addition of the precursor ALA to the cultured skin. This was necessary because, unlike the liver which demonstrates relatively high levels of porphyrin production, the skin without added ALA synthesizes practically no detectable porphyrins.

Selected concentrations of ALA (50–500 nM) were added and incubation carried out at 37° C in a humidified atmosphere of 5% CO_2 in air. Following incubation, culture medium containing the minced skin was homogenized in a Polytron (Brinkmann) and then lyophilized. Five milliliters of 1:1 perchloric acid:methanol mixture were added to the lyophilized preparation and, following filtration, the extracted samples were quantitated in a Hitachi MPF-2A fluorescence spectrophotometer using coproporphyrin as a standard. Repeated determinations of the fluorescence spectra of the porphyrins synthesized by the skin in these studies showed these to be a mixture of porphyrins consisting primarily of protoporphyrin. Therefore, data presented here are reported as pmole of protoporphyrin/mg protein since this always comprised at least 80% of the porphyrins produced by the skin. A small aliquot of the homogenized skin sample was taken for protein determination by the procedure of Sutherland et al [17] using bovine serum albumin as a standard.

Inducibility of cutaneous ALAS. In order to assess the inducibility of ALAS in the skin, two known inducers of the hepatic enzyme, allyliosopropylacetamide (AIA) and the 5β-steroid, etiocholanolone, were added to organ cultures of skin biopsies in final concentrations ranging from 1.5 to 15 nM and 5 to 50 μM, respectively. They were then incubated for 24 hr and analyzed for porphyrinogenesis as described above.

Porphyrinogenesis in the skin of patients with two types of porphyria. In these studies porphyrin production from added ALA was quantitated in skin obtained from patients with either AIP (5 patients) or PCT (6 patients). Four to eight biopsies (3- or 4-mm punches) were obtained from non-light-exposed body areas of these individuals and incubated in the organ culture system described above.

RESULTS

Porphyrin synthesis in rat and mouse skin. The Sprague–Dawley rat skin specimens incubated in tissue culture medium with ALA for 24 hr showed linear increases in porphyrin synthesis using ALA up to a final concentration of 50 μg (300 nM) (Fig. 2). Boiled skin to which ALA was added at final concentrations up to 100 μg (500 nM) showed no significant increase in porphyrinogenesis. Similarly, rat skin incubated in tissue culture medium without added ALA demonstrated no significant increase in porphyrin levels over periods up to 72 hr. The time course of porphyrin production from added ALA in rat skin is shown in Figure 3. Porphyrinogenesis began within 4 hr following the addition of ALA and there was continuous accumulation of porphyrins in a linear manner up to at least 16 hr. During the time of maximum porphyrin synthesis, removal of culture medium containing ALA and replacement with ordinary medium resulted in cessation of porphyrinogenesis.

Porphyrin synthesis in mouse skin. Skin biopsies obtained from Swiss–Webster mice also demonstrated the accumulation of porphyrins in a linear manner up to a final concentration of 300 nM of added ALA. Boiled skin with ALA, and skin specimens incubated in the absence of ALA, showed no significant porphyrinogenesis.

Porphyrin synthesis in human skin. Skin biopsies obtained from the scalps of males undergoing hair transplantation as well as the gluteal areas of normal individuals showed maximum porphyrinogenesis following the addition of ALA in concentrations identical to those that produced maximum porphyrinogenesis in rat and mouse skin (Fig. 4). This suggests that there are some similarities among the activities of the porphyrin–heme pathway in the skins of these different species.

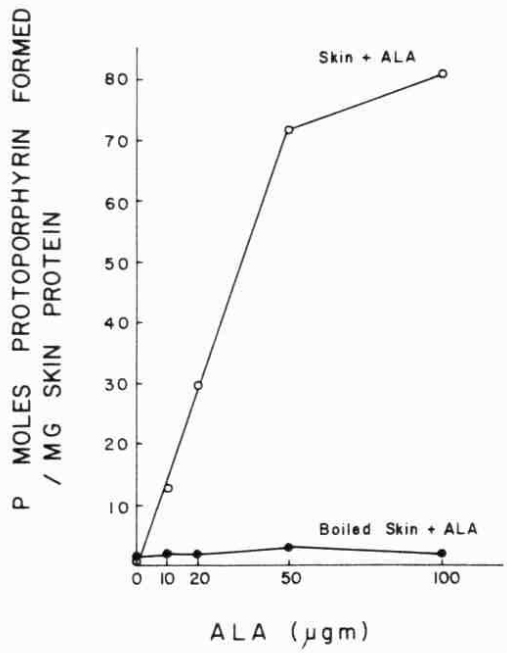

FIG. 2. Porphyrinogenesis in rat skin from added ALA. Skin samples were incubated for 24 hr with the concentrations of ALA shown and porphyrins quantitated as described in *Materials and Methods.*

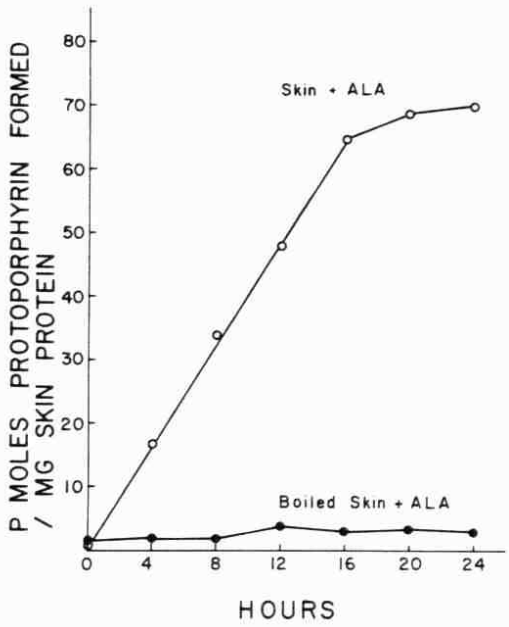

FIG. 3. Time course of porphyrinogenesis in rat skin from added ALA (100 μg). Skin samples were incubated for the time intervals shown and porphyrins quantitated as described in *Materials and Methods.*

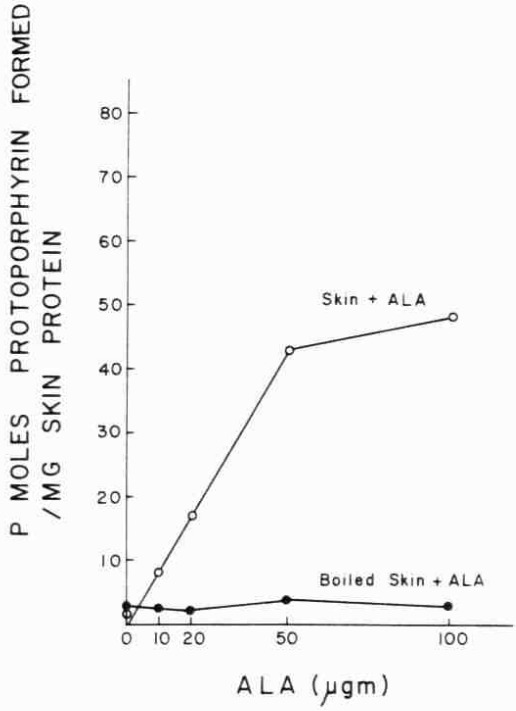

FIG. 4. Porphyrinogenesis in human skin from added ALA. Skin biopsies were incubated with ALA for 24 hr and porphyrins quantitated as described in *Materials and Methods.*

Flourescent microscopy of snap-frozen sections of skin biopsies following incubation with ALA demonstrated brilliant reddish pink fluorescence, indicative of porphyrins, to be present primarily in the epidermis or in epidermal derivatives such as

hair roots. This suggests that, under the conditions utilized in these in vitro experiments, the epidermis was the major site of cutaneous porphyrinogenesis. These data do not preclude the possibility that porphyrins synthesized in dermal components could subsequently have diffused into the epidermis.

Induction of porphyrinogenesis in rat and human skin. Addition of the hepatic porphyrinogenic drug AIA to a final concentration up to 15 nM or the 5β-steroid etiocholanolone in a final concentration up to 50 μM to the culture medium failed to elicit any greater porphyrinogenesis as compared to controls in rat and human skin samples. Our previous studies have shown that trace amounts of AIA or etiocholanolone will elicit enhanced porphyrinogenesis in chick embryo liver cells using Sassa and Granick's in vitro system [18]. These data indicate that chemical agents known to induce heme synthesis either in the liver or in the bone marrow have no demonstrable effect in the skin under the conditions of these experiments.

Porphyrinogenesis in the skin of patients with AIP and PCT. An attempt was made to confirm recent reports suggesting that there is decreased porphyrin production from precursors in cultured skin fibroblasts obtained from patients with AIP [11,12]. Skin biopsies were obtained from normal individuals and from patients with AIP and PCT. The results of these studies are shown in Figure 5. The mean value ± SE for porphyrin production from a final concentration of 500 nM ALA by human skin in 6 normal individuals was 41.6 ± 5.2 pmole protoporphyrin/mg skin protein. Studies of skin biopsies from 4 of 5 patients with AIP demonstrated a marked decrease in porphyrinogenesis to a mean value of 23.4 ± 5.9 pmole protoporphyrin/mg protein, a decrease of 43% as compared to the normal controls. In contrast, skin obtained from patients with PCT demonstrated consistently higher basal skin porphyrin levels as well as higher total accumulation of porphyrins when compared to those from normals and patients with AIP following the addition of ALA.

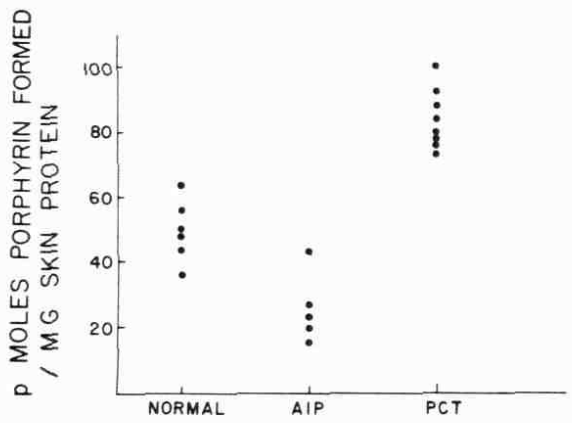

FIG. 5. Porphyrinogenesis in skin of normal individuals and patients with AIP and PCT. Skin biopsies were incubated with ALA and porphyrins quantitated as described in *Materials and Methods*.

DISCUSSION

The studies presented here indicate that mammalian skin has the enzymatic capacity to synthesize porphyrins in vitro provided that the substrate ALA is added to the tissue. This suggests that ALA formed by ALAS is rapidly converted to porphyrins and/or heme by enzymes of the heme pathway present in skin. Our data suggest the intriguing possibility that, by whatever mechanism, excessive porphyrin production in the skin itself could contribute to the elevated prophyrin levels found in patients with selected types of photosensitizing porphyria. It should be stressed that there is no unequivocal evidence for the role of skin porphyrinogenesis in cutaneous porphyria at the present time though it has been reported that intravenous infusions of ALA into human subjects resulted in mild cutaneous photosensitivity [19].

Agents known to induce ALAS activity in the liver such as AIA and the 5β-steroid etiocholanolone had no detectable effect on porphyrinogenesis in rat and human skin. This indicates that the enzyme is not inducible in the skin at least under the conditions of these studies. It is reasonable to assume that there is only a small amount of inducible ALAS activity in the skin, since cutaneous tissue does not appear to require the large amounts of heme needed by other tissues such as liver or bone marrow.

Bonkowsky et al [11] have postulated that there may be an "irreversible" type of repression of ALAS in most extrahepatic tissues of both normal and porphyric subjects. This would render the enzyme in such tissues relatively incapable of responding to exogenous drugs or chemicals. Our data in skin are consistent with that hypothesis.

Although these studies failed to demonstrate inducibility of ALAS in rat skin, it is clear from previous studies that drug metabolizing enzyme activity and the heme-protein cytochrome P-450 are inducible in the skin [20,21]. In particular, cutaneous aryl hydrocarbon hydroxylase, an enzyme which functions in the metabolism of polycyclic hydrocarbon carcinogens, is inducible (10- to 20-fold) in both rat and human skin following topical application of selected chemical agents or following incubation with the inducers in vitro [22,23]. This indicates that additional heme-protein synthesis must occur in the skin despite the fact that measurable increases in ALAS activity could not be detected in our in vitro system. Studies by DeMatteis and Gibbs [24] in rat liver have shown that the drug phenylbutazone can cause an increase in hepatic P-450 without any detectable increase in ALAS activity. There may be a pool of preformed heme present which could combine with the apo-protein for production of the complete heme-protein (in this instance cytochrome P-450). Thus, control of heme-protein

production in the skin may be dependent upon both limited ALAS activity and a pool of heme available for the production of heme-proteins.

Recent studies in the genetic disease AIP have shown that there is a deficiency of UROS, an enzyme which functions in the conversion of the monopyrrole PBG to the tetrapyrrole uroporphyrinogen I (URO I). This enzyme deficiency in AIP accounts for the excessive levels of plasma and urinary ALA and PBG found in these patients.

Skin fibroblasts of patients with AIP grown in tissue culture demonstrate deficient UROS activity [13,14] as compared to normal individuals. Data presented in this paper have shown a pronounced decrease in porphyrin synthesis by the skin of patients with AIP following the addition of ALA in vitro. Our data indicate that there is an almost 50% decrease in cutaneous porphyrinogenesis in skin biopsies of patients with AIP incubated in short-term organ culture. Studies are currently under way to assess the feasibility of measuring porphyrinogenesis from added PBG in skin since this would provide a more direct assessment of cutaneous UROS activity.

These studies have demonstrated that there is enzymatic activity in the skin capable of porphyrinogenesis from added ALA. In addition, by using a relatively simple organ culture procedure, it is possible to detect decreased porphyrinogenesis in the skin of patients with AIP which is most likely due to the UROS deficiency that has been identified in these individuals. Our findings reemphasize the fact that metabolic activity present in the skin makes this readily available tissue eminently suitable for the assessment of gene defects in human populations.

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