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THE ACTIVITY OF ARYL HYDROCARBON HYDROXYLASE IN ADULT HUMAN SKIN

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1 Aryl hydrocarbon hydroxylase (AHH), a mixed-function oxidase system, has been identified in microsomal preparations of adult breast skin and foreskin.

2 Separation of dermis from epidermis by stretching, showed that AHH activity in human skin is almost exclusively located within the epidermis.

3 Preincubation of whole chopped skin with benzanthracene (5 μM to 100 μM) using a tissue culture system was accompanied by a concentration dependent increase in AHH activity.

4 Although there was no significant difference in AHH activity between the two sites, individuals differed markedly from one another in activity at any one site. Activity was observed to be positively correlated with age.

Introduction

Aryl hydrocarbon hydroxylase (AHH) (EC 1.14.14.2), a mixed-function oxidase system, has been identified in several human tissues including liver, lungs, placenta and circulating lymphocytes and monocytes (Pelkonen, 1976). AHH activity has also been described in homogenates of human neonatal foreskin (Alvares, Kappas, Levin & Conney, 1973). Since this enzyme could play an important role in determining the activity and toxicity of drugs within the skin, we have examined some of its properties in adult skin.

Methods

Skin was obtained from surgical specimens of patients undergoing mastectomy (30 patients) or circumcision (18 patients). One mastectomized patient had been receiving primidone for several years prior to surgery and 13 were cigarette smokers. The patients' ages ranged from 7–83 years (mean ± s.e. mean 44 ± 3 years).

The skin was dissected from underlying tissues and placed in ice-cold isolation medium (20 mM tris-(hydroxymethyl)-methylamine in 0.3M mannitol at pH 7.4) at operation. After transfer to the laboratory, the subcutaneous fat was removed by scraping and the skin finely chopped with scissors. Chopped skin was homogenized in ice-cold isolation medium (15 ml) using a I.L.A. homogenizer. The homogenate was centrifuged at 10,000 *g* for 10 min and the supernatant removed and centrifuged at 100,000 *g* for 60 min. The microsomal pellet was resuspended in 50 mM Tris chloride buffer pH 7.4 containing 3 mM $MgCl_2$ (2 ml) and AHH activity measured in aliquots (0.5 ml) using the method of Nebert & Gelboin (1968) with benz(α)pyrene as substrate. Activity was expressed as pmol 3-hydroxybenz(α)pyrene (a generous gift from Dr H.V. Gelboin). The protein content of the resuspended microsomes was measured using the method of Lowry, Rosebrough, Farr & Randall (1951) and aqueous protein standards containing identical aliquots of Tris chloride buffer without dialysis.

In some experiments, the chopped skin was preincubated for 18 h in a tissue culture system (Nebert & Gelboin, 1968) at 37°C using a shaking water bath and an atmosphere of 95% O_2 and 5% CO_2. Varying quantities of benzanthracene were added to the culture medium before incubation, to give final concentrations ranging from 0 to 100 μM. Following the incubation, the samples were centrifuged at 10,000 *g* and transferred to isolation medium (15 ml) before homogenization and assay for AHH activity as described above. Preliminary experiments showed that when carried out in this manner, no fluorescent metabolites attributable to benzanthracene interfered with the AHH assay.

In four experiments, separation of dermis and epidermis was achieved by stretching. The opposite edges of specimens measuring approximately 6 cm × 4 cm were clamped, and then stretched to a tension of around 4 kg using a turn-screw attached to one of the clamps. The epidermis was removed by scraping with a scalpel, and fragments of epidermis and underlying dermis were homogenized as described above.

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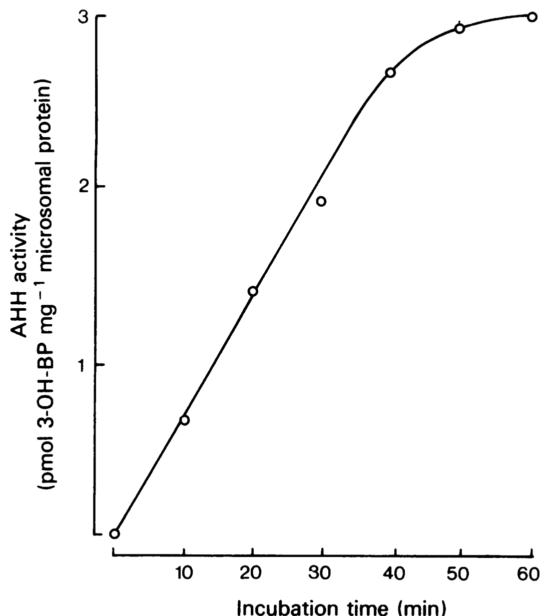

Figure 1 Time-course of the formation of phenolic metabolites of benz (α) pyrene (BP) by human skin microsomes. BP was incubated with human skin microsomes for up to 60 min at 37°C and AHH activity determined.

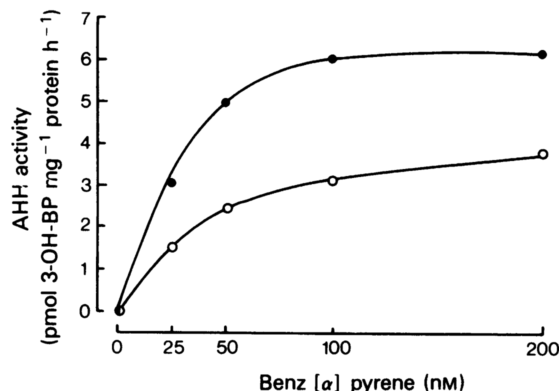

Figure 2 Graph of AHH activity (ordinate) and benz (α) pyrene concentration (abscissa). Human breast skin was cultured for 18 h in the presence (●) or absence (O) of 100 μM BA and AHH activity determined.

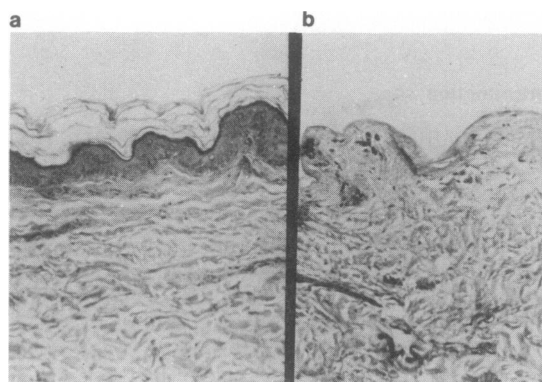

Figure 3 Photomicrographs of breast skin before (a) and after (b) removal of the epidermis.

Results

Characterization of AHH in human skin

The fluorescence spectrum of the phenolic benz(α)pyrene metabolite(s) formed by homogenates of human skin was similar to that obtained with authentic 3-hydroxybenz(α)pyrene in 1 M sodium hydroxide. The time-course of the formation of 3-hydroxybenz(α)pyrene, using varying incubation times, is shown in Figure 1. The formation of the fluorescent metabolite(s) was observed to be linearly related to the incubation time for up to 40 min. Routine assays for AHH were therefore carried out using an incubation time of 30 min, and the results were expressed as pmol 3-hydroxybenz(α)pyrene formed mg^{-1} microsomal protein h^{-1} (pmol 30H-BP mg^{-1} protein h^{-1}). The velocity of the reaction was dependent upon the substrate concentration (Figure 2) and analysis by the direct linear plot (Eisenthal & Cornish-Bowden, 1974) yielded an apparent Michaelis constant of 4.9×10^{-8} M with a V_{max} of 4.5 pmol 30H-BP mg^{-1} protein h^{-1}. AHH assays were routinely performed using benz(α)pyrene at a concentration of 100 mM.

Localization of AHH activity

Differential centrifugation of homogenized skin showed that AHH activity in the pellet obtained from centrifugation at 10,000 *g* was 0.16 pmol 3-OH-BP mg^{-1} protein h^{-1} whilst that in the pellet after centrifugation at 100,000 *g* was 4.16 pmol 3-OH-BP mg^{-1} protein h^{-1}. No AHH activity was detected in the 100,000 *g* supernatant.

Stretching the skin and removing the epidermis by scraping, produced clear separation at the dermo-epidermal junction (Figure 3) although there is clearly some contamination of the dermis with epidermal cells lying in the remnants of hair follicles. AHH activity in epidermis was 3.47 ± 0.04 pmol 3-OH-BP mg^{-1} protein h^{-1} and in dermis 0.12 ± 0.05 pmol 3-OH-BP mg^{-1} protein h^{-1}.

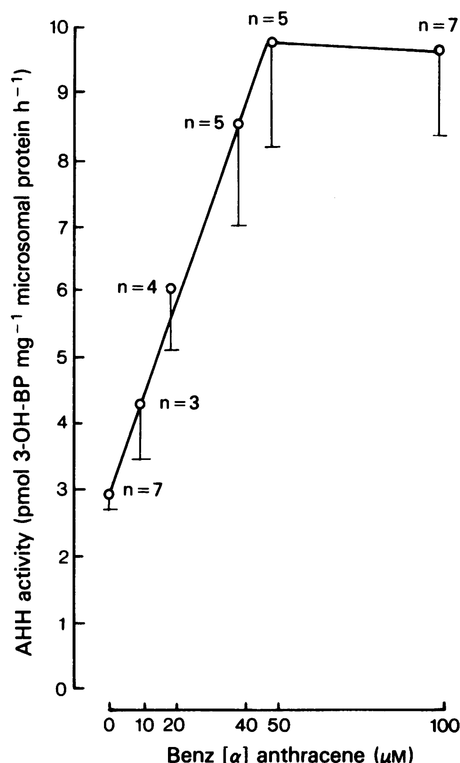

Figure 4 Change (mean ±s.e. mean) in AHH activity (ordinate) following preincubation of human skin with various concentrations of benz (*a*) anthracene (abscissa) for 18 h.

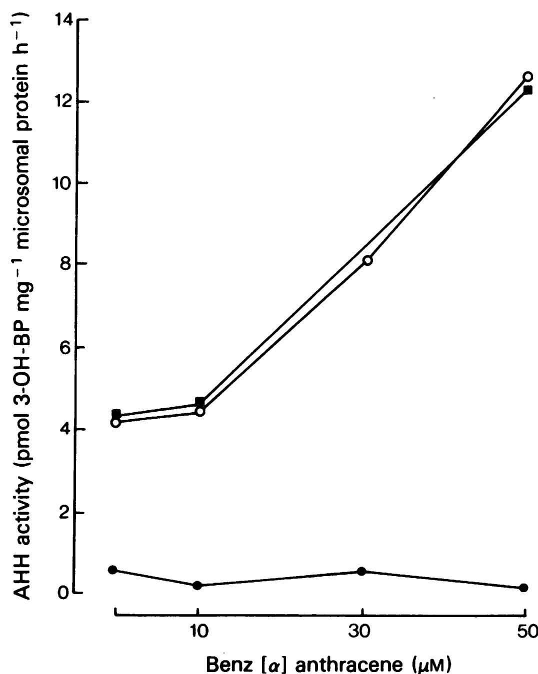

Figure 5 Change in AHH activity (ordinate) following preincubation of human skin fractions (whole skin ■, epidermis ○, and dermis ●) with various concentrations of BA (abscissa) in a tissue culture system for 18 h.

Effect of preincubation with benzanthracene

Preincubation of chopped whole skin with benzanthracene resulted in a concentration dependent increase in AHH activity (Figure 4) which reached a maximum at 50 μM benzanthracene. Preincubation of separated epidermis and dermis with benzanthracene in a single experiment (Figure 5) showed that the increase in activity was confined to the epidermis.

A double reciprocal plot of enzyme activity against substrate concentration for skin incubated with 100 μM benzanthracene yielded an apparent Michaelis constant of 4.4×10^{-8}M (see Figure 2) and a V_{max} of 8.7 pmol 3-OH-BP mg^{-1} protein h^{-1}.

AHH activity in skin

Basal AHH activity was detected in homogenates of skin in all cases. Activity was found, however, to vary widely between individuals. Thus, for breast skin activity ranged from 0.90 to 6.59 pmol 3-OH-BP mg^{-1} protein h^{-1} (mean ± s.e. mean 3.27 ± 0.21 pmol 3-OH-BP mg^{-1} protein h^{-1}). Foreskin AHH activity ranged from 1.25 to 5.07 (mean 3.19 ± 0.41) pmol 3-OH-BP mg^{-1} protein h^{-1}, and this was not significantly different from the activity in breast skin ($t=0.186$; $P>0.8$).

No difference in basal AHH activity was observed between patients who were smokers and those who were not ($t=-0.10$; $P>0.9$).

A positive correlation, however, was seen between age and basal AHH activity in breast skin ($r=+0.458$; $P<0.01$). (Figure 6.)

Seven breast skin specimens and six foreskins were incubated *in vitro* with 100–200 μM benzanthracene for 18 h and in all instances AHH activity was increased. The 'induction ratio' ('induced' activity/'basal' activity) was 3.3 ± 1.1 for breast skin and 3.5 ± 1.0 for foreskin ($t=0.155$; $P>0.5$).

Discussion

The present study confirms the existence of AHH activity in adult human skin as well as in neonatal foreskin (Alvares *et al.*, 1973). The results of the experiments in which AHH activity was examined in separated dermis and epidermis, suggests that the

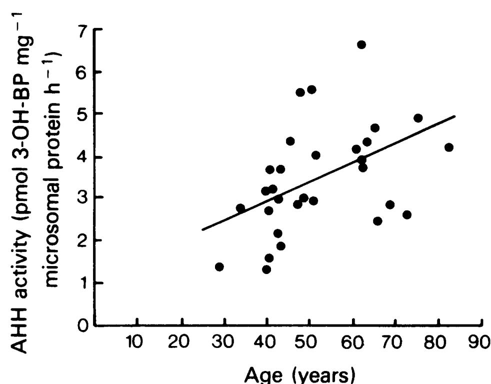

Figure 6 Relationship between basal AHH activity in breast skin (ordinate) and patients' ages (abscissa). (r=0.458, P<0.01.)

enzyme system(s) is confined to the epidermis in man: the very low levels of activity observed in separated dermis seem likely to be due to the presence of a small number of unseparated epidermal cells and hair follicles (Figure 3). As in other tissues (Nebert & Gelboin, 1968; Nebert & Gelboin, 1969) the enzyme(s) concerned with aryl hydrocarbon hydroxylation are located within the 100,000 *g* pellet of skin.

The basal activity of AHH in adult human skin appears to be of the same order of magnitude as that in neonatal foreskin (Alvares *et al.*, 1973) although the apparent Km we have observed (4.9×10^{-8}M) is lower than that reported for neonatal foreskin (4×10^{-6}M by Alvares *et al.*, 1973), for adult human liver (2.0×10^{-5}M by Alvares, Schilling, Levin, Kuntzman, Brand & Mark, 1969), or for human placenta (1.5×10^{-5}M and 2.8×10^{-5}M by Pelkonen, Jouppila & Karki, 1972). However, apparent Km values are dependent upon the conditions of measurement and the significance of these findings is therefore unknown. Our observations that AHH activity is similar in breast skin and foreskin cannot be used to infer that enzyme activity is independent of skin site until more extensive observations have been made.

Incubation of chopped skin with benzanthracene, a potent 'inducer' of AHH (Nebert & Gelboin, 1969), resulted in a concentration-dependent increase in AHH activity (Figure 4). This was associated with an increase in V_{max} but no change in apparent Km, and these findings are entirely compatible with AHH induction. The induction ratios which we have observed were similar in both breast skin and foreskin, and to those reported in neonatal foreskin (Alvares *et al.*, 1973) and in lymphocytes (Kellermann, Luyten-Kellermann, Horning & Stafford, 1976).

Basal AHH activity in adult skin shows considerable interindividual variation. Recent studies (Oesch, Schmassmann & Bentley, 1978) have shown that epoxide hydratase activity in human skin microsomes averages 18.5 nmol benzpyrene 4,5-dihydrodiol mg^{-1} protein h^{-1}. It would therefore appear unlikely that these individual differences in AHH activity reflect differences in epoxide hydratase activity. Although we have studied few subjects, we have failed to demonstrate any difference between smokers and non-smokers. Since smokers have higher levels of AHH activity than non-smokers in tissues as different as lung (Cantrell, Warr, Busbee & Martin, 1973), liver (Pelkonen, 1976) and placenta (Pelkonen *et al.*, 1972); and since the topical application of cigarette smoke condensates induces epidermal AHH in mouse skin (Norred & Akin, 1976), our results may be due to the relative inaccessibility of epidermal cells to circulating substances derived from cigarettes. Alternatively, our findings could be due to relative tissue specificity of mixed-function oxidases. However, this seems unlikely since benzanthracene appears to induce AHH activity in human skin. The present study suggests that basal AHH activity is positively correlated with age. This contrasts with the age-related decline in hepatic microsomal hydroxylation which has been described in man for some drugs (Crooks, O'Malley & Stevenson, 1976).

Assuming that AHH activity is similar in all skin sites, and that human skin weighs approximately 4 kg, we estimate that the total capacity of skin to metabolize benz(α)pyrene amounts to 9.7 nM/min. By contrast, total human liver metabolizes approximately 131.7 nM/min. It therefore seems unlikely that skin metabolism could play a major role in overall drug metabolism in man. However, skin metabolism of drugs could prove to be important where there is impaired hepatic function for other substrates.

AHH may, however, be important in the therapeutic efficacy and toxicity of topical drugs. In particular, dithranol and coal tars are effective remedies for the treatment of psoriasis (Bettley, 1963) and both are polycyclic hydrocarbons. Their pharmacological effects may, therefore, be at least partially determined by epidermal AHH activity. Moreover, AHH may be important in determining the cytotoxicity and carcinogenicity of some foreign compounds (Gelboin, 1977) and could possibly be involved in the pathogenesis of skin tumours.

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