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Phenylethanolamine N-Methyltransferase-like Immunoreactivity in Psoriasis

An Immunohistochemical Study on Catecholamine Synthesizing Enzymes and Neuropeptides of the Skin

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Immunoreactivity for phenylethanolamine N-methyltransferase (PNMT), the enzyme involved in the conversion of norepinephrine to epinephrine, was present in the basal epidermis and upper dermis in 16 patients with psoriasis. The amount of immunoreactivity was increased tenfold in involved compared to uninvolved skin as characterized by computer-assisted image analysis. In skin from healthy volunteers no immunoreactivity could be found. In our subjects, no immunoreactivity was observed for the other catecholamine synthesizing enzymes (tyrosine hydroxylase; dopa-decarboxylase; dopamine-β-hydroxylase), apart from single tyrosine hydroxylase positive adrenergic vascular nerves. Furthermore, in psoriasis, the immunoreactivity pattern of the peptides somatostatin, substance P, vasoactive intestinal polypeptide and bombesin was in agreement with skin from healthy volunteers. *Key words: Human; Noradrenaline N-methyltranferase: Peptides; Immunohistochemistry.* (Received June 5, 1986.)

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Studies on peptides using radioimmunoasssay and/or immunohistochemistry have been done in conditions such as symptomatic cutaneous mastocytoma (1), acanthosis nigricans (2), urticaria pigmentosa (3), diabetic lipodystrophy (4), and lichen sclerosus et atrophicus (5). In psoriatic skin no unique peptide immunoreactivity has so far been observed (6). It has been shown that the epidermis possesses a catecholamine sensitive site, which can activate adenylate cyclase and result in cAMP accumulation within the epidermal cells (7). Furthermore, in psoriasis a reduced cAMP/cGMP ratio has been reported (8). Finally, activity of the catecholamine synthesizing enzymes dopa-decarboxylase (DDC) and dopamine-β-hydroxylase (DBH) has been observed in the human skin (9, 10).

Against this background we decided to investigate the presence of the four catecholamine synthesizing enzymes as well as of some peptides in patients with psoriasis. Immunofluorescence on thin tissue sections using antibodies against the various compounds was performed (11) and the size of the obtained patterns was morphometrically quantified, using computer-assisted image analysis (11, 12), and expressed as a volume density value based on planimetric profile-area percentage measurements.

MATERIALS AND METHODS

Subjects

Sixteen patients (9 males) with stationary plaque psoriasis, with a mean age of 43 years (range: 17–74 years), a mean duration of disease of 14 years (range: 4 months-37 years), and a mean duration of the

current relapse of 3½ months (range: ½–8 months), were investigated. One patient was treated with methyldopa (500 mg/day) and metoprolol (400 mg/day) due to hypertension, another patient received levothyroxine (0.15 mg/day). The other patients had no oral medication. The current relapse had not been treated with any topical therapy except for three patients who had been treated with low concentrations of dithranol at 1 or 2 occasions more than one week before biopsy. Six healthy volunteers (3 males), mean age 30 years (range: 24–37 years), without psoriasis heredity or a history of psoriasis, were used as controls. The present study was approved by the Committee of Ethics at the Karolinska Hospital.

Antisera

Antibodies to tyrosine hydroxylase (TH; 13), dopa-decarboxylase (DDC; 14), dopamine-β-hydroxylase (DBH; 15), phenylethanolamine N-methyltransferase (PNMT; 14), somatostatin (16), substance P (17), vasoactive intestinal polypeptide (VIP; 18, 19) and bombesin (20) were generated in rabbits or rats. As control sera, either normal serum (for the catecholamine synthesizing enzymes) or antiserum absorbed with an excess of the respective immunogen (50 μg/ml antiserum diluted 1: 10; 2–4 h at room temperature during continuous shaking or 12–24 h at +4°C) (for the peptides) was used. The antisera used showed no cross-reactivity with other substances in radioimmunological or immunohistochemical tests. However, cross-reactivity with known, not tested substances, or with unknown peptides or proteins present in the tissue section, with a chemical composition similar to PNMT, for example, cannot be excluded and therefore terms such as 'PNMT-like immunoreactivity' have been considered appropriate in the text.

Preparation of tissue

Punch biopsies (3 mm) were taken under local anaesthesia with lidocaine without epinephrine from involved and uninvolved (minimum distance to involved skin 4 cm) gluteal skin of the patients and from gluteal skin of the controls. After immersion for 2–4 h in 0.02 M para-benzoquinone in 10% fresh formalin, the tissue pieces were rinsed for at least 24 h in 0.1 M Sörensen's buffer containing 10% sucrose, 0.01% NaN_3 and 0.02% Bacitracin (Sigma Chemical Co., St. Louis, Mo., USA), sectioned perpendicularly on a cryostat (section thickness 14 μm) and processed for indirect immunohistochemistry as described previously in detail (11).

Immunohistochemistry

The sections were incubated, with the antisera diluted (TH: 1:400; DDC: 1:200; DBH: 1:400; PNMT: 1:800; somatostatin: 1:400; substance P: 1:10; VIP: 1:400; bombesin: 1:200; control sera: corresponding dilutions) in 0.01 M phosphate buffered saline (PBS), over-night at +4°C in a humid atmosphere, rinsed in PBS for 10 min with 3 changes, then incubated for 30 min at +37°C with fluorescein-isothiocyanate (FITC)-labeled anti-rabbit or anti-rat serum (Amersham International plc, Amersham, U.K., and Dakopatts, Copenhagen, Denmark, respectively) diluted 1: 10, rinsed as before and mounted in glycerine/PBS (10: 1) containing 0.1% para-phenylenediamine. As an additional control, certain sections were only incubated with the second antiserum. The sections were studied and photographed in a Zeiss fluorescence microscope with a dark-field oil condenser and a HBO 200 high pressure mercury lamp with a 3 or 4 mm Schott BG12 or a KP500 as excitation filter and a Zeiss 50 or LP520 as barrier filter. Following photography, the cover slips were removed and, after rinsing, certain sections were stained with hematoxylin-eosin for routine histology.

Quantitative morphometry

10 sections from each patient (5 from involved skin and 5 from uninvolved; double measurements per section) were analyzed by a commercially available semiquantitative interactive image analysis system (IBAS, Zeiss/Kontron), consisting of a host computer (IBAS 1) and an image processing unit (IBAS 2). The images were investigated using a black and white TV-camera. First, the image was digitalized and stored in the IBAS 2. A pseudo-colour transformation of the image grey scale revelaed the fluorescence intensity distribution in the section (11). Enhancement of contrast and sharpness was made, thereafter a grey level threshold was interactively set to select for the cellular material excluding most of the background in the specimens. This threshold was kept constant in all measurements. After generation of the binary picture and median filtering to remove 'noise' elements, remaining non-specific background elements and artefactual areas in the field of view were excluded. The cellular structures were selected for measurement of the amount of immunoreactive material using the area % (=size of the obtained patterns calculated as a planimetric profile area and expressed, in relation to the reference area, as the field-specific profile-area percentage value) which was then converted to a volume density value. For details see Johansson (11) and Johansson & Hallman (12).

RESULTS

Immunoreactivity to PNMT (Fig. 1), TH, substance P and VIP was found in the skin of the patients investigated. A cytoplasmic, sometimes more granular, PNMT-like immunoreactivity was present in round, oval or spindle-shaped cellular structures within the involved as well as the uninvolved epidermis (mainly basal layers) and dermis (mainly upper parts) of the psoriatic patients (Fig. 1). However, there was a considerable difference in number of PNMT immunoreactive structures between the two types of patient skin, i.e. involved skin contained much more of positive elements than uninvolved (compare Fig. 1 A–C with 1 D). There were also differences between the patients regarding the overall staining intensity, i.e. some patients exhibited a strong immunoreactivity whereas others showed a less strong staining pattern. Morphometric analysis of the PNMT immunoreactive cellular structures resulted in a volume density, calculated from the area fraction, of 4.52 (0.36) % (mean (S.E.M.)) in involved and 0.63 (0.003) % in uninvolved skin, i.e. in an area of 1 cm^2 of involved skin there would be 16 mm^3 of immunoreactive tissue and in 1 cm^2 of uninvolved skin the corresponding value would be 1.6 mm^3, assuming a mean thickness of 3.5 mm in involved skin and 2.5 mm in uninvolved skin. It should be noted, that the patient on methyldopa and metoprolol medication had volume density values which did not differ significantly from the group means. Neither did the volume density values of the three patients treated with low concentrations of dithranol a few times at least a week before biopsy (clinically judged as irrelevant to disease severity in the single lesion) differ significantly from the means. In skin from healthy volunteers virtually no PNMT-like immunoreactivity could be found.

Furthermore, the pattern seen for TH and the neuropeptides was not changed in the psoriasis patients compared to normal skin, i.e. single TH positive adrenergic vascular nerves, some few substance P nerve fibers in the dermis (sometimes close to the epidermis) as well as a plexus of VIP positive nerve fibers around blood vessels and close to sweat glands, sebaceous glands and hair follicles of the dermis and subcutis could be seen, which is supported by earlier observations in man and in several types of experimental animals (for Refs., see 21 and 22).

After incubation with control sera none of the specific fluorescent structures described above could be observed. However, after incubation with blocked antiserum or only with the second, FITC-labeled, antiserum some diffuse general 'background staining' of a low intensity was seen in the connective tissue fiber network as well as a strong unspecific reaction in the upper epidermis (cf. Fig. 1).

DISCUSSION

PNMT is normally found in adrenergic neurons of the nervous system as well as in endocrine cells where it is the known enzyme responsible for the conversion of norepinephrine to epinephrine (for Refs., see 23). So far no PNMT immunoreactivity has been observed with certainty in normal skin. To our knowledge, however, this investigation is the first observation of PNMT-like immunoreactivity in psoriatic skin. The PNMT-like immunoreactivity was, in addition to the localization to dermal cells, observed in cells of mainly the basal layer of the epidermis. The reason for not seeing any PNMT-like immunoreactivity in normal epidermal or dermal cells may be dependent on too low levels of the enzyme and/or it being loosely bound in the normal cells.

The hypothesis that a defect in the adenylate cyclase-guanylate cyclase system is an important etiological factor in psoriasis has been suggested by Voorhees et al. (8). Epidermal cells possess a catecholamine receptor coupled to adenylate cyclase (7), and this receptor is probably β-adrenergic (24), possibly of the β_2 subclass (25). It has been

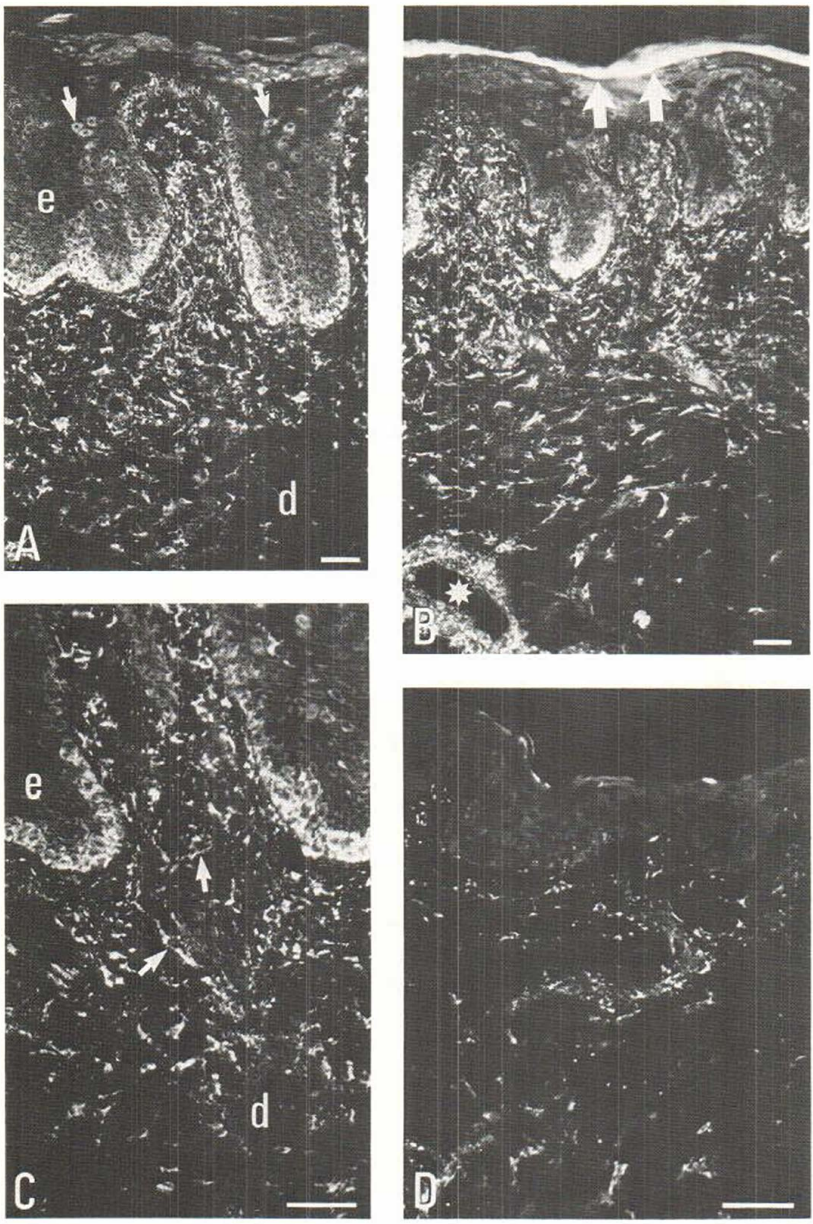

Fig. 1 A–D. Low (A, B) and high (C, D) power immunofluorescence micrographs of involved (A–C) and uninvolved (D) psoriatic skin after incubation with antiserum to PNMT. Note the great difference between the involved and the uninvolved skin regarding the amount of immunoreactivity for PNMT. Also note the immunoreactivity found in cells of the epidermis (*e*) and dermis (*d*) (arrows in A and C, respectively). Asterisk in B denotes blood vessel surrounded with a large number of positive structures. Unspecific background staining was found in e.g. the stratum corneum (arrows in B) and in the connective tissue network of the dermis. Bars indicate 50 μm.

shown, that basal cells are more responsive to epinephrine than more superficial cell layers (26). Epinephrine is more effective than norepinephrine in inducing epidermal cAMP accumulation in the epidermis (7). This effect leads to the inhibition of epidermal proliferation (27).

On a hypothetical basis, several explanations for our findings could be proposed. If epidermal proliferation is assumed to be caused by some other factor(s), the increase in PNMT-like immunoreactivity could reflect increased conversion of norepinephrine to epinephrine as a secondary attempt to decrease proliferation. If, on the other hand, the increase in PNMT-like immunoreactivity is regarded to reflect a primary phenomenon, at least two explanation could be suggested: (1) Epinephrine is produced to inhibit keratinocyte proliferation via adenylate cyclase, but the β-adrenergic receptor is defect. In fact, a reduced responsiveness of adenylate cyclase to epinephrine in psoriasis plaques has been reported (28); (2) The enzyme is produced but it is functionally defect, and therefore lacks normal activity. Increased production of a defect enzyme could be expected as a feed-back mechanism. It should be pointed out, that in this study we have only investigated the ability of the antiserum to discover antigenic sites of the enzyme (i.e. *presence* of the enzyme or a related substance in the tissue), and not the enzymatic *activity*.

In this study, the PNMT-like immunoreactivity was confined to cellular structures, sometimes with a more granular appearance. Only future ultrastructural immunocytochemical studies can give a final answer to the exact cell type(s) responsible for the pattern of immunoreactivity as well as to the subcellular localization of PNMT in these cells. However, it should be noted that neither the hematoxylin-eosin stained sections nor the distribution pattern of the PNMT-like immunoreactivity gave the impression that these cellular structures represented neurons. Interpreting these immunohistochemical findings, we should call in mind the observations of Adams-Ray & Nordenstam (29). They demonstrated granulated, chromaffin cells in the upper dermis, mainly in close connection to blood vessels. However, the existence of an extra-neuronal store of catecholamines in human skin has been questioned (30).

Further immunochemical studies are now in progress where we intend to investigate the existence of PNMT (or a related substance) in other dermatoses. In this context, it could be noted that the antibodies used do not cross-react with histamine N-methyltransferase (M. Goldstein, unpublished results), an enzyme normally found in high amounts in the skin (cf. Brown et al. (31)). Furthermore, Imamura et al. (32) could not show any increase in histamine N-methyltransferase activity in psoriatic skin. Of course, this does not rule out the possibility of a structural change in the latter enzyme, due to the disease conditions, which could result in a cross-reaction between the enzyme and the PNMT-directed antibodies.

It has recently been shown, that psoriatics react upon mental stress with increased epinephrine secretion in the urine compared to healthy, stressed controls (33). This finding suggests that stress, which is a known trigger factor in psoriasis, produces a stronger activation of the sympato-adrenomedullary system in psoriatics. PNMT is the enzyme responsible for the production of epinephrine in the adrenal medulla. Whether similar stress regulation of skin PNMT (or a structurally related molecule) occurs, and whether it is altered in psoriatics, remains to be investigated.

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