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Cytokines and glucocorticoids differentially regulate APN/CD13 and DPPIV/CD26 enzyme activities in cultured human dermal fibroblasts

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Abstract Cultured human dermal fibroblasts coexpress two cell surface ectopeptidases, aminopeptidase N (APN/CD13) and dipeptidyl peptidase IV (DPPIV/CD26). These enzymes catalyze the removal of a single amino acid or a dipeptide from the N-termini of oligopeptides, respectively. They are also localized in a differential pattern in normal, non-sun-exposed, adult skin, a finding that supports the supposition that these enzymes might have different functions in the skin, but relatively little is known about their functions in the skin. A better understanding of how the activities of these enzymes are regulated should increase our understanding of their functions in the skin. APN/CD13 was routinely expressed at higher levels on cultured fibroblasts than was DPPIV/CD26. Treatment of cultured fibroblasts with specific factors differentially modulated the activities of these enzymes. APN/CD13 was significantly upregulated by treatment with interleukin-4 (IL-4), interferon γ (IFN γ), and the glucocorticoids dexamethasone and hydrocortisone. In contrast, the regulation of DPPIV/CD26 activity was found to be different and more complex. This enzyme was consistently upregulated by IL-1 α and IL-1 β , but consistently downregulated by glucocorticoids, tumor necrosis factor α (TNF α) and transforming growth factor β_1 (TGF β_1). Thus, although these two enzymes are expressed on the same populations of cultured cells, their activities are differentially regulated. This finding, along with their differential distribution in normal skin, suggests that APN/CD13 and DPPIV/CD26 have different functions in the skin.

Keywords Dermis · Fibroblast · Aminopeptidase N · Dipeptidyl peptidase IV

Introduction

Normal adult human skin contains multiple populations of integral membrane peptidases. These ectopeptidases include neutral endopeptidase (NEP/CD10, EC 3.4.24.11), which is homologous to CD10, aminopeptidase N, which is homologous to CD13 (membrane alanyl aminopeptidase, APN/CD13, EC 3.4.11.2), and dipeptidyl peptidase IV, which is homologous to CD26 (DPPIV/CD26, EC 3.4.14.5) (Look et al. 1989; Ulmer et al. 1990; De Meester et al. 1999; Olerud et al. 1999; Riemann et al. 1999). In normal adult skin, NEP/CD10 is primarily associated with keratinocytes (Scholzen et al. 1998, 2001; Olerud et al. 1999), although it has also been identified on the surface of cultured human fibroblasts (Kletsas et al. 1998). In contrast, APN/CD13 and DPPIV/CD26 are primarily associated with dermal fibroblasts (Verlinden et al. 1981; Saison et al. 1983; Raynaud et al. 1992; Bou-Gharios et al. 1995; Piela-Smith and Korn 1995; Stefanović et al. 1998; Sorrell et al. 2003). Immunohistochemical and histochemical studies of human skin have determined that these latter two enzymes are localized to the dermis. Furthermore, studies have shown that APN/CD13 protein and enzyme activity, but not DPPIV/CD26 protein and enzyme activity, appear at sites of epithelial/mesenchymal interactions in skin (Sorrell et al. 2003).

The activities of APN/CD13 and DPPIV/CD26 appear to be regulated by the amount of enzyme at the surface of cells (Riemann et al. 1995; Kehlen et al. 1998). Previous studies have shown that the levels of message for APN/CD13 and DPPIV/CD26 in cultured renal carcinoma cells are regulated by specific growth factors/cytokines. In dermal fibroblasts, it has been shown that interleukin 4 (IL-4) and dexamethasone both increase the activity of APN/CD13 (Piela-Smith and Korn 1995; Stefanović et al. 1998). Less is known about other factors that might regulate the activities of APN/CD13 and DPPIV/CD26 present on the surface of human dermal fibroblasts. An understanding of these factors should provide insight into the function of the enzymes in the skin.

Previous studies have shown that enzymatically active APN/CD13 and DPPIV/CD26 are coexpressed on the sur-

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face of cultured human dermal fibroblasts (Raynaud et al. 1992; Bou-Gharios et al. 1995; Sorrell et al. 2003). The purpose of this study was to identify and compare factors that regulate the activities of APN/CD13 and DPPIV/CD26 on cultured human dermal fibroblasts that express both of these enzymes. For this purpose, three populations of human dermal fibroblasts were compared: fibroblasts obtained from full-thickness fetal trunk skin and adult fibroblasts derived from the papillary dermis of two normal adult donors. The results of these studies confirmed the coexpression of these two enzymes on the surface of normal human fibroblasts. Furthermore, the two enzymes were differentially regulated by the addition of growth factors/cytokines and glucocorticoids. The activity of APN/CD13 was upregulated by the T-cell-derived cytokines IL-4 and interferon γ (IFN γ) and also by the glucocorticoids dexamethasone and hydrocortisone. In contrast, the activity of DPPIV/CD26 was upregulated by the proinflammatory cytokines IL-1 α and IL-1 β ; however, other proinflammatory cytokines such as IL-6 and tumor necrosis factor α (TNF α) either had no effect or downregulated the activity of DPPIV/CD26. Transforming growth factor β_1 (TGF β_1) also downregulated the activity of DPPIV/CD26.

Thus, even though these two enzymes were coexpressed by the same populations of cultured fibroblasts their activities were differentially regulated. This feature, combined with their differential location in human skin implies that APN/CD13 and DPPIV/CD26 have different functions in the skin. These results do not identify the functions of these two ectopeptidases in the skin. However, previous studies have suggested that such peptidases may act to regulate the amount of neuropeptides in the skin (Pincelli et al. 1993; Scholzen et al. 1998; Wallengren 1999).

Methods

Sources of fibroblasts

Three human dermal fibroblast populations were compared in these studies. Fetal dermal fibroblast cultures were initiated from samples obtained from the Central Laboratory for Human Embryology at the University of Washington (Seattle, Wash.). These samples were samples of full-thickness trunk skin obtained from of a 145-day estimated gestational age fetus (Sorrell et al. 1999). Dr. Thomas McCormick (Department of Dermatology, Case Western Reserve University) provided a strip of skin dermatomed at a depth of 0.4 mm from the buttock region of a 41-year-old consenting donor with normal skin. The keratinocyte layers from the fetal skin and the dermatomed skin samples were enzymatically detached and the remaining dermal tissue was treated with trypsin and collagenase prior to mechanically dissociating the tissue to obtain primary cellular suspensions. The cells obtained in this manner are referred to as fetal and donor-1 cells in the text. Adult donor-2 cells were provided by Dr. Irwin Schafer (Department of Pediatrics, Case Western Reserve University). Skin was dermatomed from the trunk region of the cadaver of a 36-year-old individual (Schafer et al. 1985). Fibroblast cultures from this sample were obtained through explant culture. All tissue samples were obtained from either consenting donors or from discarded tissue. All human studies were reviewed by the Institutional Review Board at Case Western Reserve University and were therefore performed in accordance with the ethical standards laid down in an appropriate version of the 1964 Declaration of Helsinki.

Cell culture

Dermal fibroblasts were grown as adherent cultures on plastic culture dishes using Dulbecco's modified Eagle's Medium (DMEM; Sigma Chemical Company, St. Louis, Mo.) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Rockville, Md.). The culture medium was replaced three times weekly. All fibroblasts used for these studies were in either their fourth or fifth passage. Typically, cells were plated at low density (2100 cells/cm²) onto 35-mm tissue culture dishes and cultured for the indicated times (see below).

Cytokine treatment of cultures

Experimental cultures were treated with recombinant human cytokines IL-1 α , IL-1 β , IL-6 and IL-8, dexamethasone and hydrocortisone (all obtained from Sigma). IFN γ was obtained from Boehringer Mannheim, and IL-4 and soluble IL-6 receptor (sIL-6R), human recombinant TGF β_1 and human recombinant TNF α were all obtained from R & D Systems (Minneapolis, Minn.). Pilot studies (data not shown) indicated that maximal changes in enzyme activity occurred by day 7 of cytokine treatment. Thereafter, cytokines were routinely added to DMEM supplemented with 2% FBS for 2, 4, and 7 days before assaying for enzyme activity. However, only the 7-day data are presented here. Maximal changes resulting from glucocorticoids occurred by day 4. The concentrations of the cytokines/growth factors used for the study were (ng/ml): IL-1 α , 1; IL-1 β , 1; IL-4, 1; IL-6, 15; sIL-6R, 15; IL-8, 15; TGF β_1 , 2.5; TNF α , 10; IFN γ , 100. The concentrations of the glucocorticoids used were (μ M): dexamethasone, 1; and hydrocortisone, 0.83.

Assays for enzymatic activities on cultured dermal fibroblasts

The quantitative assays for APN/CD13 and DPPIV/CD26 on cultured human dermal fibroblasts were performed according to previously published methods (Raynaud et al. 1992). In brief, all assays were performed, in triplicate, on cells adherent to 35-mm plastic culture dishes (Falcon, Franklin Lakes, N.J.). The buffer used for the assays was the same as that used by Raynaud et al. (1992). The substrates used for the APN/CD13 assays were 2 mM alanine *p*-nitroanilide (Ala *p*-NA), 2 mM arginine *p*-nitroanilide (Arg *p*-NA), and 2 mM proline *p*-nitroanilide (Pro *p*-NA), and the substrates for the DPPIV/CD26 assays were 2 mM glycine-proline *p*-nitroanilide (Gly-Pro *p*-NA) and 2 mM arginine-proline *p*-nitroanilide (Arg-Pro *p*-NA), all obtained from Sigma.

After removing the culture medium and washing the culture plates with a physiologic saline solution, 1 ml substrate was added and the culture dishes were incubated at 37°C for 30 min. At that point, an aliquot of incubation medium was removed from the culture dish and added to stop buffer (Raynaud et al. 1992). Free *p*-nitroaniline was measured spectrophotometrically at 405 nm and was compared to a standard curve of *p*-nitroaniline (Sigma). After measuring enzyme activities, the fibroblasts were detached from the culture dishes with 0.5% trypsin/1 mM sodium ethylenediaminetetraacetic acid solution (Life Technologies) and the number of cells on each plate was determined by hemocytometer counts. The activity was expressed as nanomoles *p*-nitroaniline produced per minute by 1×10^5 cells. Bestatin (Sigma) and diprotin A (Sigma) were combined with substrates at a level of 1 mM for each inhibitor. Bestatin is a known inhibitor of APN/CD13 activity and diprotin A (Ile-Pro-Ile) is a known inhibitor of DPPIV/CD26 activity (Raynaud et al. 1992; Piela-Smith and Korn 1995; Nemoto et al. 1999).

Statistical analysis

The paired two sample *t*-test for means was used to determine whether two experimental values were significantly different, with *P* values <0.05 taken as indicating significance.

Results

APN/CD13 and DPPIV/CD26 activities on unstimulated dermal fibroblasts

Previous studies have shown that the APN/CD13 and DPPIV/CD26 ectoenzymes metabolize substrates substi-

tuted with different peptides at different rates. APN/CD13 prefers peptide substrates with alanine at the N-terminus, but also acts upon other substrates with different amino acids at the N-terminus, although at slower rates (Raynaud et al. 1992; Bou-Gharios et al. 1995; Piela-Smith and Korn 1995). In this study, three substrates were compared: Ala p-NA, Arg p-NA, and Pro p-NA (Fig. 1). The highest

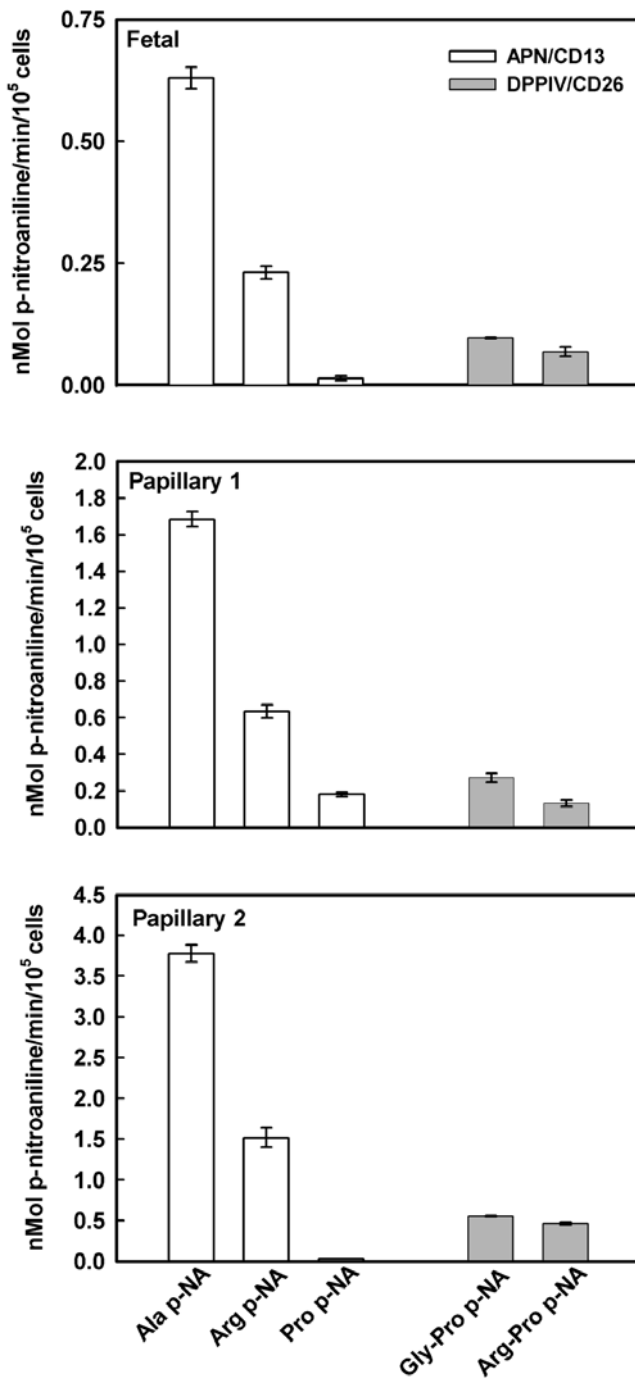


Fig. 1 Comparison of APN/CD13 and DPPIV/CD26 activities on different substrates. The activities of APN/CD13 on three different substrates (Ala p-NA, Arg p-NA, Pro p-NA), and of DPPIV/CD26 on two different substrates (Gly-Pro p-NA, Arg-Pro p-NA) were compared. *Error bars* represent standard deviation of the mean

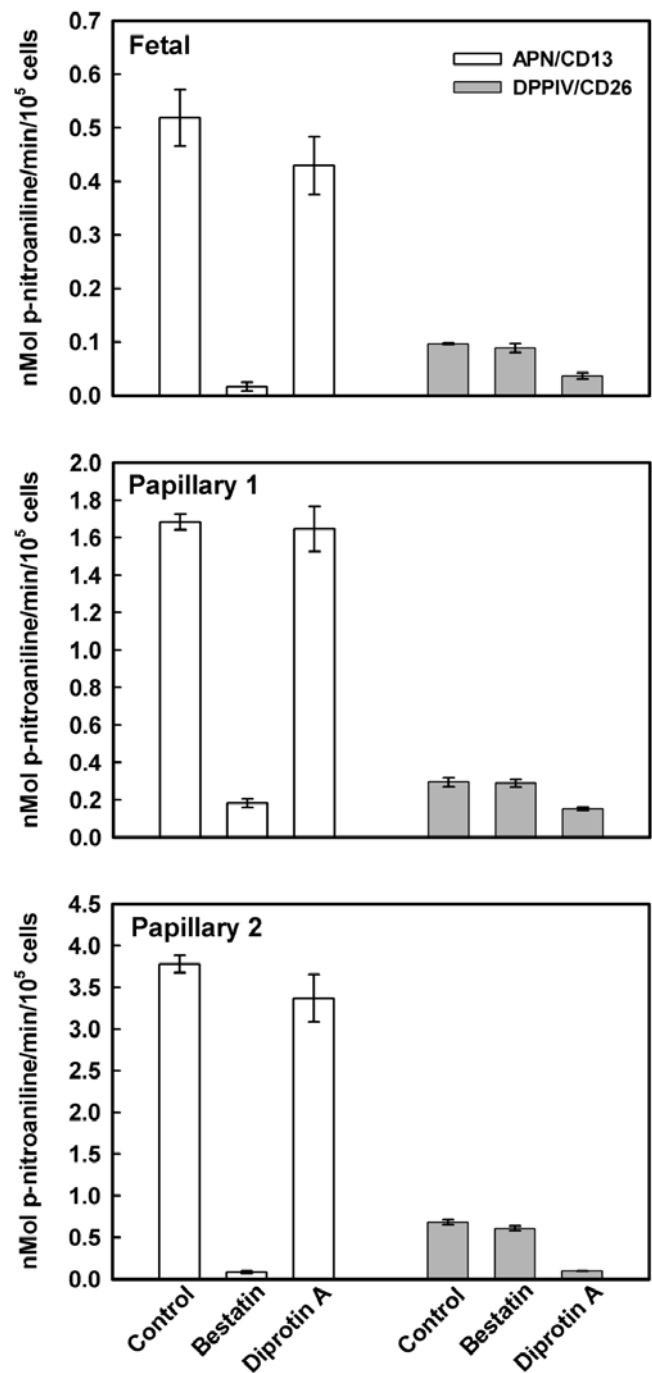


Fig. 2 Effects of inhibitors on APN/CD13 and DPPIV/CD26 activities. Activities of APN/CD13 and DPPIV/CD26 were assayed in the presence of Ala p-NA or Gly-Pro p-NA, respectively. The inhibitors bestatin and diprotin A, both at 1 mM, were combined with these two substrates. *Error bars* represent standard deviation of the mean

Table 1 Enzymatic activity of different dermal fibroblasts plated at high density and cultured for 7 days. The values presented are representative of multiple experimental assays

^aThe substrate used was Ala p-NA

^bThe substrate used was Gly-Pro p-NA

Cell population	Enzymatic activity (nmol <i>p</i> -nitroaniline/min/10 ⁵ cells)	
	APN/CD13 ^a	DPPIV/CD26 ^b
Fetal (145 days estimated gestational age)	0.465±0.012	0.0806±0.0013
Adult papillary dermis		
Donor 1	2.91 ±0.085	0.715 ±0.014
Donor 2	1.77 ±0.13	0.508 ±0.024

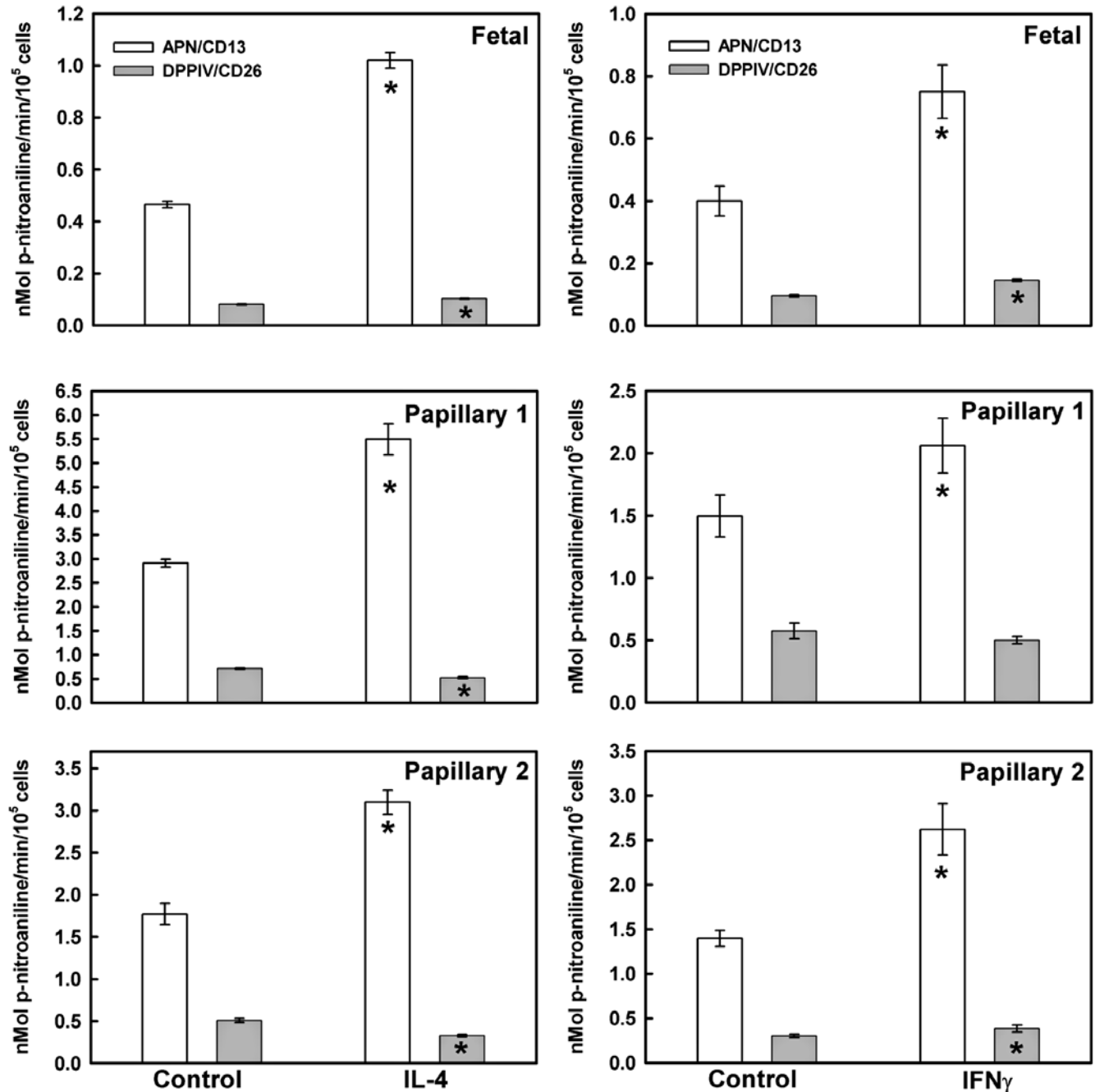


Fig. 3 IL-4 and IFN γ and APN/CD13 and DPPIV/CD26 activities. Fetal fibroblasts and two populations of adult papillary dermal fibroblasts (*Papillary 1*, *Papillary 2*) were treated with IL-4 or IFN γ for 7 days prior to assaying for enzyme activities. The rela-

tive enzyme activities of treated cells are presented, and the values shown are the means of three experiments. Error bars represent standard deviation of the mean. * $P < 0.05$ compared to control

levels of enzyme activity occurred with the Ala p-NA substrate. Similarly, DPPIV/CD26 prefers substrates with a glycine-proline dipeptide located at the N-terminus, but also catalyzes the removal of other dipeptides at slower rates (Raynaud et al. 1992; Bou-Gharios et al. 1995; Piela-Smith and Korn 1995). In this study, Gly-Pro p-NA and Arg-Pro p-NA substrates were compared (Fig. 1). Enzyme activity was higher when the Gly-Pro p-NA substrate was present.

The specificities of the enzymatic reactions were also tested by inclusion of known inhibitors of APN/CD13 and DPPIV/CD26. APN/CD13 activity is selectively inhibited by bestatin whereas DPPIV/CD26 activity is selectively inhibited by diprotin A (Raynaud et al. 1992; Bou-Gharios et al. 1995; Piela-Smith and Korn 1995; De Meester et al. 1999; Riemann et al. 1999). Figure 2 shows the effects of these two inhibitors on enzyme activities on their preferred substrates. Inclusion of bestatin with the substrate resulted in an approximately 90% or greater inhibition of APN/CD13 activity; however, addition of diprotin A had at best only a modest effect on enzyme activity. In contrast, inclusion of bestatin did not affect DPPIV/CD26 activity, but inclusion of diprotin A resulted in an approximately 50% or greater inhibition. Similar inhibitory effects were demonstrated for all three cell populations and are consistent with those found in other studies (Raynaud et al. 1992; Bou-Gharios et al. 1995; Piela-Smith and Korn 1995; Nemoto et al. 1999).

Three different dermal fibroblast populations, one from a fetal donor and two from adult donors, were compared for their abilities to degrade the Ala p-NA and Gly-Pro p-NA substrates of APN/CD13 and DPPIV/CD26, respectively (Verlinden et al. 1981; Saison et al. 1983; Raynaud et al. 1992; Bou-Gharios et al. 1995; Nemoto et al. 1999). Fetal and adult dermal fibroblasts expressed the activities of both enzymes (Table 1). The two adult dermal fibroblast populations expressed comparable, though not completely identical, levels of enzyme activity. In each case, APN/CD13 activity expressed on a per cell basis was significantly higher than that of DPPIV/CD26. In contrast, the activity on fetal fibroblasts was five- to eightfold lower than on adult fibroblasts.

Effect of different cytokines and growth factors on the activity of APN/CD13 and DPPIV/CD26

The T-cell-derived cytokines IL-4 and IFN γ normally have antagonistic effects (Craven et al. 2001). However, prior studies have indicated that both of these cytokines might enhance APN/CD13 activity (Riemann et al. 1995; Kehlen et al. 1998). This possibility was tested for the three human dermal fibroblast cell populations described above. Maximal changes in enzyme activity were observed after 7 days of treatment with the various cytokines. Thereafter, cultures were routinely treated with IL-4 and IFN γ for 2, 4, and 7 days. No significant modifications in enzyme activity were observed after 2 days and intermediate levels of change were observed after 4 days. Therefore,

the results obtained for 7 days of treatment are shown. As shown in Fig. 3, treatment of cells with IL-4 or with IFN γ significantly increased APN/CD13 activity. DPPIV/CD26 activity was differentially modulated by these two cytokines in a more complex manner. Treatment with IL-4 significantly increased DPPIV/CD26 activity on fetal dermal cells, but suppressed its activity on adult cells (Fig. 3). Treatment with IFN γ increased enzyme activity on fetal cells and on one of the two sets of adult cells (Fig. 3).

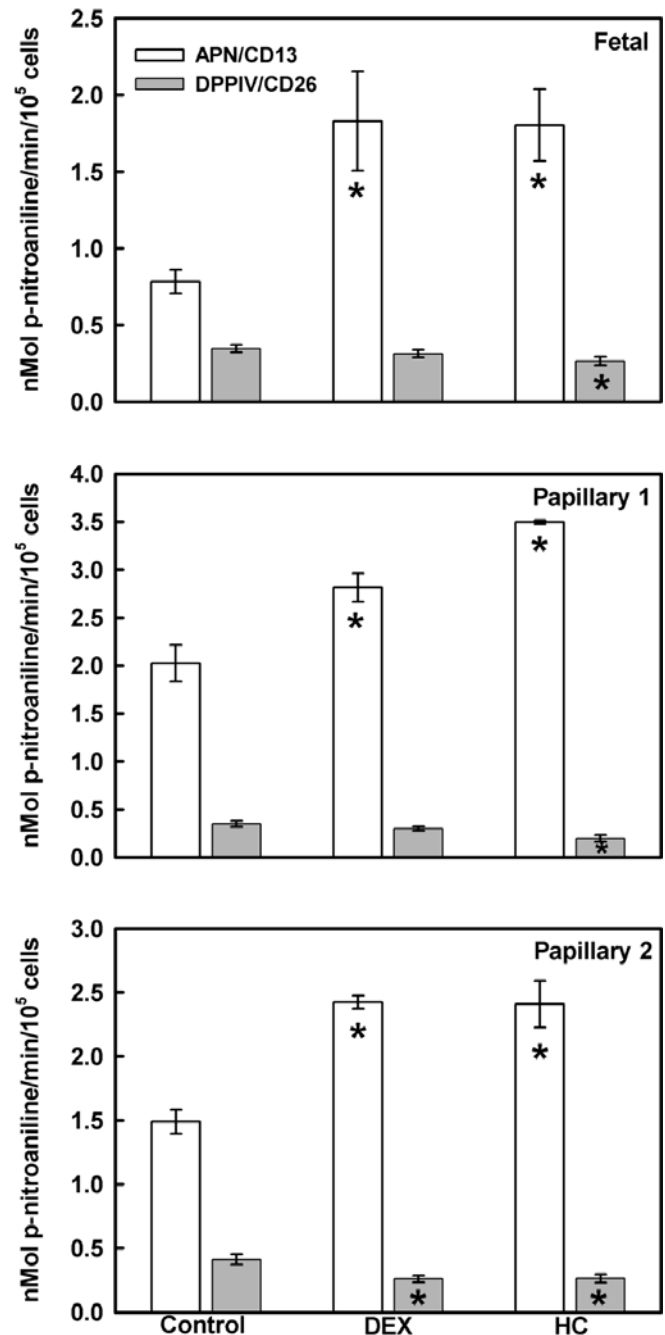


Fig. 4 Dexamethasone and hydrocortisone and APN/CD13 and DPPIV/CD26 activities. Dermal fibroblasts were treated with dexamethasone (DEX) or with hydrocortisone (HC) for 4 days prior to assaying for enzyme activities. Error bars represent standard deviation of the mean. * $P < 0.05$ compared to control

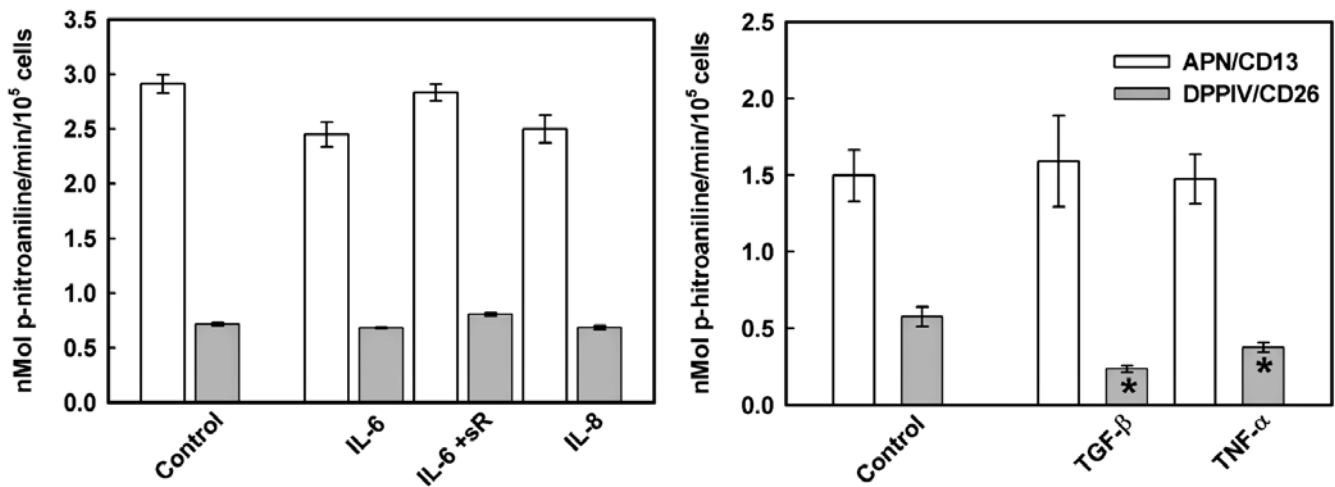


Fig. 5 IL-6, IL-6 plus sIL-6, IL-8, TNF α , and TGF β ₁ and APN/CD13 and DPPIV/CD26 activities. Adult papillary dermal fibroblasts were treated with the indicated growth factors/cytokines 7 days prior to assaying for enzyme activities. Error bars represent standard deviation of the mean. * $P < 0.05$ compared to control

Both dexamethasone and hydrocortisone significantly increased APN/CD13 activity after 4 days of treatment (Fig. 4). Dexamethasone suppressed DPPIV/CD26 activity on only one set of adult cells, but did not significantly change the activity on fetal cells or on the other set of adult cells (Fig. 4). Hydrocortisone treatment suppressed enzyme activity in all three populations of cells (Fig. 4).

Treatment of cultured adult dermal fibroblasts with IL-6, with or without soluble receptor (Boxman et al. 1996; Mihara et al. 1996; Liechty et al. 2000), induced no significant modulation of the activities of either APN/CD13 or DPPIV/CD26. The chemokine IL-8 and the growth factors TNF α and TGF β likewise did not significantly alter APN/CD13 activity on cultured human dermal fibroblasts (Fig. 5). However, both TNF α and TGF β suppressed DPPIV/CD26 activity. Although not shown, similar results were obtained for the fetal dermal cells and the second set of adult papillary dermal cells.

The proinflammatory cytokines IL-1 α and IL-1 β did not regulate APN/CD13 activity on fetal or on one set of adult cells (Fig. 6), but produced a modest, but significant, reduction in activity on the other set of adult cells. However, these cytokines increased DPPIV/CD26 activity in all three fibroblast populations (Fig. 6).

Discussion

The cell surface ectopeptidases APN/CD13 and DPPIV/CD26 appear on multiple cell types and in a wide assortment of tissues (De Meester et al. 1999; Riemann et al. 1999). In human skin, these ectoenzymes are primarily expressed on the surface of fibroblasts (Saison et al. 1983; Raynaud et al. 1992; Bou-Gharios et al. 1995; Piela-Smith and Korn 1995; Sorrell et al. 2003). Coexpression of these two ectopeptidases on cultured human dermal fi-

broblasts has been demonstrated by flow cytometric studies (Sorrell et al. 2003). In this study, the regulation of enzyme activity on cultured human dermal fetal and adult fibroblasts was assayed after exposure of cultured cells to cytokines and other factors that might potentially modulate these enzyme activities (Piela-Smith and Korn 1995; Riemann et al. 1995; Kehlen et al. 1998; Stefanović et al. 1998). The results of these studies indicated that the activities of APN/CD13 and DPPIV/CD26, while coexpressed on surfaces of cultured human dermal fibroblasts, were nonetheless expressed at different levels and are differentially regulated. Furthermore, these studies also provide evidence that the presence and regulation of enzyme activities on the surface of fetal dermal cells differed from that on adult dermal cells.

Previous studies have shown that APN/CD13 and DPPIV/CD26 activities are regulated at the level of transcription and that there are no known tissue inhibitors or cofactors that might regulate their activities on the cell surface (Riemann et al. 1995; Kehlen et al. 1998; De Meester et al. 1999; Riemann et al. 1999). Studies have shown that cultured mesenchymal and epithelial cells regulate the activities of these enzymes in response to specific cytokines (Table 2 summarizes these studies). However, there have been only a limited number of studies identifying factors that regulate enzyme activities on the surface of human dermal fibroblasts. IL-4 and dexamethasone have been shown to increase the activity of APN/CD13 (Piela-Smith and Korn 1995; Stefanović et al. 1998), a feature that was confirmed in the present study. Also, the current study demonstrated that APN/CD13 activity was regulated by IFN γ and hydrocortisone.

There are no known studies in which the regulation of DPPIV/CD26 activity on the surface of human dermal fibroblasts has been investigated, and there are only a limited number of studies in which the regulation of DPPIV/CD26 activity on other cell types has been investigated. Studies of DPPIV/CD26 expression by cultured renal epithelial cells (Table 2) indicate similarities with these findings. Both IL-4 and IFN γ regulate DPPIV/CD26 activity on skin fibroblasts and on renal epithelial cells. Also, TNF α and TGF β both suppress DPPIV/CD26 activity on dermal fi-

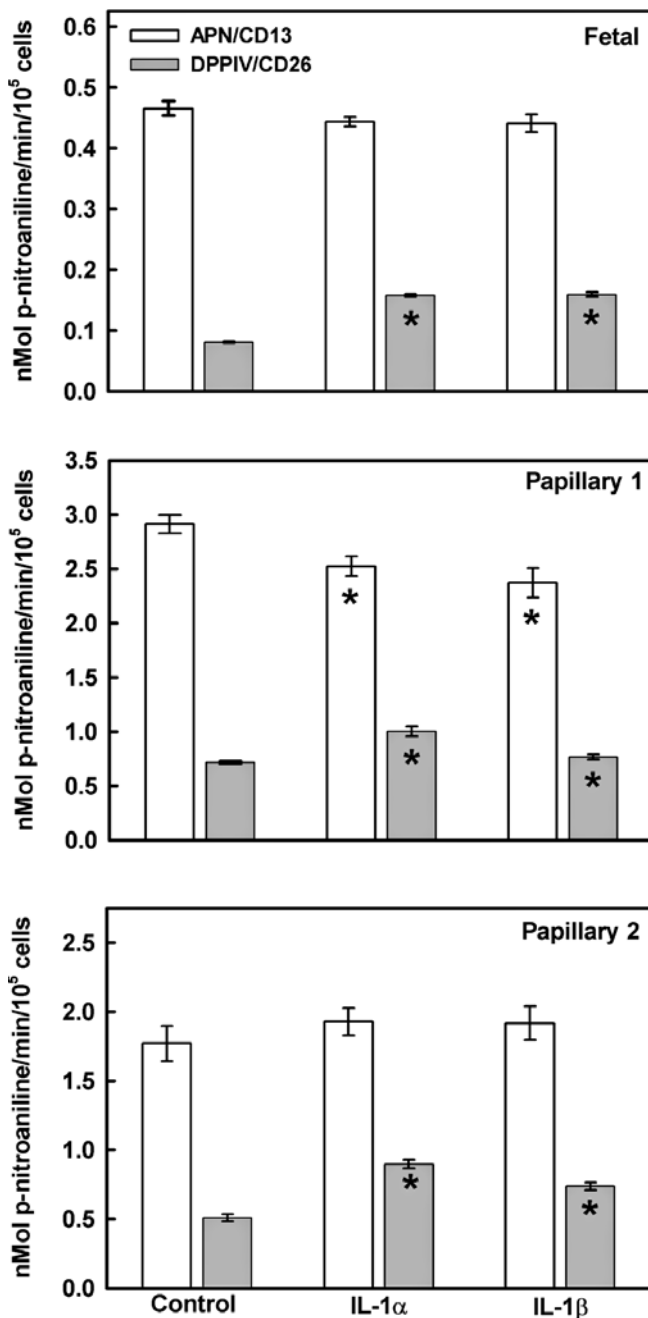


Fig. 6 IL-1 α and IL-1 β and APN/CD13 and DPPIV/CD26 activities. Fetal and adult papillary dermal fibroblasts were treated with IL-1 α or IL-1 β for 7 days prior to assaying for enzyme activities. Error bars represent standard deviation of the mean. * $P < 0.05$ compared to control

broblasts and on renal carcinoma cells. In addition, these studies have provided novel evidence that DPPIV/CD26 activity on dermal fibroblasts is regulated by IL-1 (enhanced) and by glucocorticoids (suppressed).

The functions of ectopeptidases in skin have yet to be established. However, clues as to their putative functions can be obtained from their locations in skin and the nature of their natural substrates. Two of these ectopeptidases NEP/CD10 and APN/CD13 are primarily expressed in the

epidermis and the dermal epidermal-junction regions, respectively (Olerud et al. 1999; Sorrell et al. 2003). These locations correspond to sites where unmyelinated nerves terminate and release neuropeptides such as substance P, calcitonin gene-related protein, and neurokinin A (Pincelli et al. 1993; Schulze et al. 1997; Scholzen et al. 1998; Wallengren 1999). These neuropeptides have potent vasoactive properties and promote the migration of inflammatory cells into the skin (Scholzen et al. 1998; Wallengren 1999). All three of these neuropeptides are potential substrates for NEP/CD10, and neurokinin A is also a potential substrate for APN/CD13 (Russell et al. 1996; Scholzen et al. 1998; Wallengren 1999). In addition, IL-8, which also mediates the recruitment of leukocytes into skin, is another potential substrate for APN/CD13 (Kanayama et al. 1995; Riemann et al. 1999; Uchi et al. 2000). Thus, NEP/CD10 and APN/CD13 may cooperate in regulating the amounts and activities of neuropeptides and chemokines in the superficial layers of skin. DPPIV/CD26 also regulates the activities of neuropeptides as well as chemokines such as RANTES, eotaxin, and LD78 β (Hoffmann et al. 1993; Oravec et al. 1997; Proost et al. 1998; 2000, Mentlein 1999; Struyf et al. 1999). However, its differential location in the skin, combined with its differential regulation, suggests that it operates independently of the other two ectopeptidases.

APN/CD13 protein and enzyme activity consistently appear at sites of epithelial/mesenchymal interactions. In human skin, elevated levels of enzyme occur in the dermal papillae of hair follicles as well as in the dermal-epidermal junction region (Sorrell et al. 2003). A distinctive association of APN/CD13 with epithelial tissue has also been observed in fetal human breast tissue (Atherton et al. 1994a, 1994b). This pattern of localization suggests that this enzyme might play a role in epithelial/mesenchymal interactions. Again, such interactions might be related to neuropeptides. Neuropeptides have been shown to play a role in the regulation of hair growth (Peters et al. 2001; Castex-Rizzi et al. 2002). The presence of APN/CD13 in the dermal papillae of follicles may regulate the levels of these factors.

Previous studies have demonstrated that fetal dermal fibroblasts are physiologically distinct from adult dermal fibroblasts (Cullen et al. 1997; Liechty et al. 2000). The studies presented here provide additional evidence for the physiologic diversity of fetal and adult dermal fibroblasts. The most striking difference between fetal and adult dermal fibroblasts is the absence of DPPIV/CD26 in fetal and neonatal skin, where neither the protein nor the enzyme activity can be detected. Similar results have also been obtained for the expression of DPPIV/CD26 in developing human breast tissue (Atherton et al. 1994a, 1994b). Primary and low-passage fetal dermal fibroblasts do not express DPPIV/CD26, but this surface antigen is acquired when these cells have been cultured for longer periods (Atherton et al. 1994b). In this study, activities of both APN/CD13 and DPPIV/CD26 were detected on cultured fetal dermal fibroblasts, but the levels of activity for both enzymes were significantly less than that on adult dermal fibroblasts.

Table 2 Regulation of APN/CD13 and DPPIV/CD26 enzymatic activities by living human non-myeloid cells (ND not determined, ↑ increase, ↓ decrease, ≈ no change, ? not statistically significant)

Cytokine/growth factor	Cell type	Duration of exposure	Effect on APN/CD13	Effect on DPPIV/CD26	Reference
IL-1 α 10 ng/ml	Gingival fibroblasts	6 days	Activity ↑	ND	Nemoto et al. 1999
IL-1 α 10 ng/ml	Periodontal ligament fibroblasts	6 days	Activity ≈	ND	Nemoto et al. 1999
IL-1 β 17 ng/ml	Osteosarcoma cells	1 day	mRNA ↑	ND	Russell et al. 1996
IL-1 β 5 ng/ml	Renal epithelial cells	3 days	Activity ≈ mRNA ≈	Activity ≈	Riemann et al. 1995
IL-1 β 20 ng/ml	Bronchial epithelial cells	1 and 2 days	Activity ≈	ND	Riemann et al. 1995
IL-4 100 U/ml	Dermal fibroblasts	Overnight	Activity ↑	ND	Piela-Smith and Korn 1995
IL-4 2 ng/ml	Renal epithelial cells	3 days	Activity ↑ mRNA ↑	Activity ↑	Riemann et al. 1995
IL-4 20 ng/ml	Bronchial epithelial cells	1 and 2 days	Activity ≈	ND	Riemann et al. 1995
IL-6 1 nM	Osteosarcoma cells	1 day	mRNA ↑	ND	Nemoto et al. 1999
IL-6 500 U/ml	Renal epithelial cells	3 days	Activity ≈	Activity ≈	Riemann et al. 1995
IFN γ 200 U/ml	Renal cell carcinoma	1 day	mRNA ↑?	mRNA ↑	Kehlen et al. 1998
TGF β 10 ng/ml	Renal cell carcinoma	1 day	mRNA ↑?	mRNA ↓	Kehlen et al. 1998
TNF α 100 U/ml	Renal cell carcinoma	1 day	mRNA ↑?	mRNA ↓	Olerud et al. 1999
Dexamethasone 1 μ M	Dermal fibroblasts	3 days	Activity ↑	ND	Stefanović et al. 1998

Thus, fetal dermal cells differ from adult dermal cells primarily in their overt levels of active enzyme. Otherwise, fetal cells resemble their adult counterparts in the regulation of enzyme activities.

In summary, these results indicate that even though the two cell surface ectopeptidases APN/CD13 and DPPIV/CD26 are coexpressed by cultured fetal and adult human dermal fibroblasts, their *in vitro* levels of expression are differentially regulated by exogenous factors. This feature, along with the differential distribution of enzyme activity in the skin, suggests that each enzyme has a unique function in skin physiology. Nonetheless, this does not preclude the possibility that under certain conditions these two enzymes may function in a cooperative manner.

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