Journal of Cellular Biochemistry 43:243–254 (1990)

Neutral Endopeptidase From Nuchal Ligament of Fetal Calves

Alice R. Johnson, Lynn D. Gray, Elky Youngblood, and James Sullivan

Departments of Biochemistry and Cell Biology, University of Texas Health Center at Tyler, Tyler, Texas 75710

The nuchal ligament of unborn calves contains a neutral endopeptidase that is biochemically and immunologically similar to the neutral endopeptidase (NEP), or enkephalinase, from human kidney. Enzymatic activity was inhibited more than 90% by phosphoramidon (1 μM). The specific activity in membrane fractions, as determined by hydrolysis of the dansylated substrate, DAPGN, was similar in tissue from fetuses of gestational ages ranging from 100 to 280 days. NEP activity in adult ligament tissue, however, was less than 10% of that in fetal tissue. Fibroblasts dissociated from ligament tissue by collagenase displayed less NEP activity than did preparations of intact ligament, and activity was even lower in cultured cells. By contrast, fibroblasts cultured from fetal calf lungs had NEP activity comparable to that in the ligament tissue. When ligament fibroblasts were cultured on subcellular matrices derived from fetal lung fibroblasts the NEP activity increased relative to those cultured on plastic alone. These studies confirm the presence of neutral endopeptidase (NEP) in the nuchal ligament of the fetal calf. The consistent activity through a range of gestational ages and the influence of the subcellular matrix suggest that this enzyme might be involved in growth of the ligament during fetal life.

Key words: neutral endopeptidase, nuchal ligament, cultured fibroblasts

Neutral endopeptidase (NEP), or enkephalinase (E.C.3.4.24.11), is localized on microvillus structures of epithelia in kidney, intestine, male reproductive organs, and placenta [1]. Human polymorphonuclear neutrophils [2–4] and cultured human fibroblasts contain a similar enzyme [5,6]. Recently the common acute lymphocytic leukemia antigen (CALLA) was identified as NEP [7,8].

NEP inactivates a variety of biologically active peptides. The list of naturally occurring substrates with diverse physiologic activities includes enkephalins, substance P, oxytocin, kinins, neurotensin, atrial naturetic factor, interleukin 1-β, and the chemotactic peptide, fMet-Leu-Phe [1]. The range of substrates for NEP appears to be far more diverse than previously suspected, and the physiologic function of this enzyme probably depends primarily on its location.

In earlier studies with fibroblasts from human tissues, we found much higher NEP activity in fibroblasts from skin than in those from lung [5]. The high enzymatic activity

Received September 1, 1989; accepted February 21, 1990.

in fibroblasts from neonatal and fetal skin suggested that it might influence cellular interactions involved in growth and differentiation, possibly by modifying peptide signals.

We recently reported that the nuchal ligament of unborn calves contains neutral endopeptidase activity [9]. This tissue, which is composed of a morphologically homogeneous population of fibroblasts, has been studied as a model for elastin gene expression during fetal life [10]. In this study we measured NEP in ligaments taken at various gestational ages to determine if activity changed during gestation. We also evaluated the influence of an extracellular matrix on expression of NEP activity in cultured cells.

MATERIALS AND METHODS

Extraction of Neutral Endopeptidase Activity

Ligaments were dissected from fetal calves immediately after removal from the uterus. Gestational age was estimated by crown to rump measurement, and the isolated ligament was placed in tissue culture medium (RPMI) containing 0.1 mM of freshly prepared phenylmethylsulfonylfluoride (PMSF) (BRL) at 4°C; 30 g of tissue was weighed, minced finely with scissors, and homogenized in a Waring blendor at 4°C in 150 ml of 50 mM MES [2-(N-morpholine)ethanesulfonic acid] buffer (pH 6.5) containing 0.1 mM PMSF. The homogenate was centrifuged for 5 min at 1,000g at 4°C. The supernatant was reserved and the material in the pellet was homogenized again with MES buffer and centrifuged and both supernatants pooled.

The pooled supernatant fractions were passed through a sintered glass Millipore filter and centrifuged at 100,000g for 1 h to collect a membrane fraction. The membranes were resuspended in 10 mM Tris HCl (pH 7.5) containing 0.1 mM PMSF and 0.5% CHAPS and left at 4°C overnight to solubilize the enzyme. Insoluble material was removed by centrifugation at 100,000g for 1 h; 1–5 ml of the CHAPS extract was applied to a 10/10 (10 cm × 10 mm) mono Q FPLC column and fractionated using a 0–500 mM NaCl linear gradient containing 10 mM Tris HCl and 0.5% CHAPS. The peak of activity eluted at a concentration of ~250 mM NaCl. The active fractions were pooled and concentrated in an Amicon apparatus using a YM 30 membrane. NEP was extracted and partially purified from frozen human kidney tissue by the same method for comparison with the ligament enzyme. Protein was measured by the method of Bradford [11].

Enzymatic Activity

Activity of NEP in tissues and cells was measured by the method of Florentin et al. [12] in a continuous recording assay. The fluorogenic substrate, N-dansyl-D-alanyl-glycyl-p-nitrophenylalanylglycine (DAGNPG) (Sigma) in a final concentration of 50 μM in 50 mM Tris HCl (pH 7.5), was cleaved to release p-nitrophenylalanylglycine. Fluorescence at 562 nm was measured in a Perkin-Elmer photofluorometer using an excitation wavelength of 342. The NEP inhibitor, phosphoramidon (1 μM) was added to parallel samples in each assay to establish the specificity of the reaction.

Immunoblotting of Ligament and Renal NEP

Immunoreactivity with an antibody to human renal NEP was determined to ascertain that the enzymatic activity from the fetal calf ligament corresponded to that in

earlier preparations from this laboratory [9] and others [13,14]. The proteins in the active peak fractions of FPLC preparations from ligament and kidney were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), using a Canalco gel system. Samples (150 μg) were applied in duplicate to 10% gels and run under nonreducing conditions. Molecular weight markers were used to estimate Mr of the protein bands. Proteins in the completed gel were transferred to nitrocellulose paper using 100 V for 60 min in a BioRad Trans-Blot system. The blot was divided into two parts and incubated either with antihuman NEP (a generous gift from Dr. E.G. Erdös, University of Illinois at Chicago) or with normal rabbit serum. The samples were then washed and incubated with a goat antirabbit antibody conjugated to alkaline phosphatase. The reaction was developed in 100 mM Tris HCl (pH 9.5) containing 100 mM NaCl, 5 mM $MgCl_2$, and alkaline phosphatase substrates, NBT (50 mg/ml) and BCIP (50 mg/ml) dissolved in 70% and 100% dimethylformamide [15].

Cell Culture

Ligaments and lung tissues were dissected from the fetus using aseptic conditions and washed through several changes of RPMI 1640 medium containing antibiotics. The tissue was finely minced with scissors, and small pieces (1–2 mm^3) were placed in T-25 culture flasks and covered with medium containing fetal calf serum (FCS) (10%) and antibiotics. Cells migrated from the explants within 24 h, but the tissue was left in place for 72 h. Once several patches of cells were established, the tissue was removed and the adherent cells covered with fresh medium. Medium was changed three times per week, and cells were passed when the monolayer reached 80–90% confluency. Cells in second to fifth passage were used for experiments.

In some experiments, fibroblasts were dissociated from fresh ligament tissue by collagenase. Finely chopped tissue was incubated in a solution of 0.5% tissue culture collagenase (GIBCO) in a shaking water bath for 1 h at 37°C. The dissociated cells were collected by filtration through sterile gauze, centrifuged, washed twice in PBS containing 1% FCS, and counted in a Coulter electronic cell counter. Aliquots were reserved for cell culture; the remainder of the cells were pooled to provide a membrane preparation for NEP determination.

Extracellular Matrix Preparations from Cultured Cells

Fibroblasts from either nuchal ligament or fetal bovine lung were cultured on 100 mm (GIBCO) tissue culture plates. Cultures were initiated with 200,000 cells per plate; 7 days after the monolayers became confluent, the cells were lysed by addition of distilled water containing 0.1 mM PMSF and freezing and thawing several times. For comparison, some plates were prepared by removal of the cells with a solution of 1 mM EDTA in Puck's saline. The cell-free matrix was then treated with DNAse I (Sigma) 20 μg/ml for 60 min at 37°C, washed twice with 5 mM EDTA in sterile PBS, and frozen at −20°C until used in experiments. Matrices prepared by either distilled water lysis or EDTA gave comparable results.

Phase and Fluorescent Microscopy

Cultured fibroblasts were plated on tissue culture chamber slides (Lab-Tek) for immunocytochemistry. The cells were fixed in 100% methanol for 10 min at room temperature. The slides were washed twice in Hank's buffered salt solution containing

TABLE I. Enzymatic Activities From Fetal Calf Ligament and Human Kidney*

Fraction	NEP activity (μmol/h/ml)	Protein (mg/ml)	Specific activity (μmol/mg)
Kidney			
Crude membranes	620	1.9	326
Mono Q fraction	345	0.1	3,450
Ligament			
Crude membranes	500	1.2	416
Mono Q fraction	525	0.2	2,763

*Enzyme preparations are described in the text. Crude membranes were sedimented at 100,000g for 1 h and extracted with CHAPS (0.5%). Mono Q fractions were active peaks collected by FPLC of membrane extracts.

10% fetal calf serum (HBSS–FCS), the appropriate primary antibodies were added at a dilution of 1:100, and the slides were incubated in a moist chamber for 60 min at room temperature. Antivimentin, a monoclonal antibody, was from Boerhringer-Mannheim, and anticytokeratin, a rabbit polyclonal antibody, was from Biomedical Technologies, Inc. Rabbit polyclonal antihuman renal NEP is described above. The slides were washed three times with HBSS–FCS, and the secondary antibody (either fluorescinated goat antirabbit IgG or fluorescinated goat antimouse IgG) was applied and incubation was continued for 30 min at room temperature. They were washed three times with HBSS–FCS, once with PBS, mounted and photographed in phase and fluorescence modes on an Olympus Vanox-T light microscope using a 20× Olympus S PLAN objective.

Transmission Electron Microscopy

Pieces of nuchal ligament were fixed in a solution of 3% glutaraldehyde buffered with 0.1 M sodium cacodylate and postfixed in 1% buffered OsO_4. The samples were dehydrated in ethanol and embedded in epoxy resin. Semithin sections of approximately 0.5 μm were examined by light microscopy to select areas for thin sectioning. Thin sections of ≤100 nm in thickness were mounted on copper grids, viewed, and micrographed in a Siemens 101 transmission electron microscope.

RESULTS

Neutral Endopeptidase Activity in Calf Ligament

Membrane preparations from fetal calf ligament contained NEP activity as determined by assay with the dansylated substrate, DAGNPG [12]. Inhibition by phosphoramidon discriminated NEP from other peptidases in the preparations [1]. Table I compares the specific activities of crude membrane fractions isolated from fetal calf ligament and human kidney and the specific activities after fractionation by FPLC. These initial isolation steps provide comparable amounts of activity from either tissue.

FPLC separation of material extracted from the membrane fractions resulted in a single active peak that eluted in the linear gradient at ~250 mM NaCl. The peak activity from either calf ligament or kidney preparations eluted from the mono Q column in the same fractions. This material was purified approximately 10-fold relative to the mem-

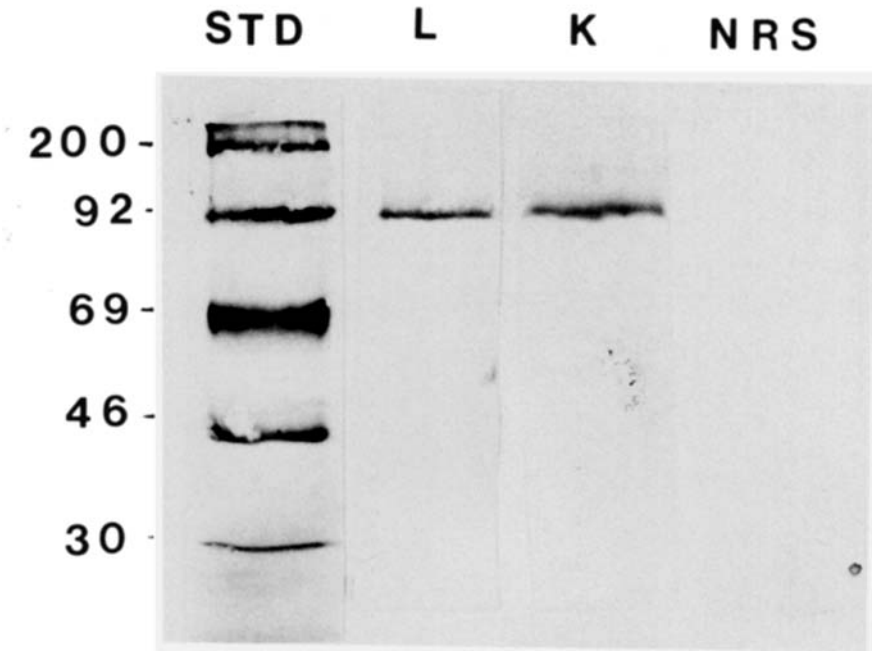

Fig. 1. Western blot of NEP from ligament (L) or kidney (K). The FPLC active peaks (100 μg) from either preparation were concentrated and separated in 10% nonreduced SDS–polyacrylamide gels. Rabbit antihuman renal NEP recognizes a band of ~92,000 M_r in either preparation. Normal rabbit serum (NRS) was unreactive.

brane fraction (Table I). Enzymes from either source were more than 90% inhibited by 1 μM phosphoramidon.

The ligament enzyme was further indentified by immunoreactivity with a polyclonal antibody against human renal NEP. Figure 1 shows a Western blot of the active peaks derived from FPLC separation of the crude membrane extracts. A single major band with ~92,000 M_r was recognized by the rabbit polyclonal antibody to human renal NEP in both ligament and kidney samples. None of the separated proteins reacted with normal rabbit serum.

Enzymatic Activity at Different Gestational Ages

Elastin formation in the nuchal ligament has been well characterized by Mecham and Senior [10], and accumulated evidence indicates that elastogenesis begins at a discrete stage in gestation. In contrast to reported changes in elastin [10], we found no consistent change in the specific activity of NEP in the nuchal ligament over a range of gestational ages from 100 days to 280 days (Fig. 2). The specific activities of NEP in membrane preparations from fetal ligaments ranged from 415 to 610 μmol/h/mg. By contrast, NEP activity in ligaments from mature animals ranged from 9 to 40 μmol/h/mg.

Fibroblasts from Nuchal Ligament

Electron microscopy of nuchal ligaments showed a uniform population of cells embedded in a connective tissue matrix composed primarily of collagen (Fig. 3a,b). Both

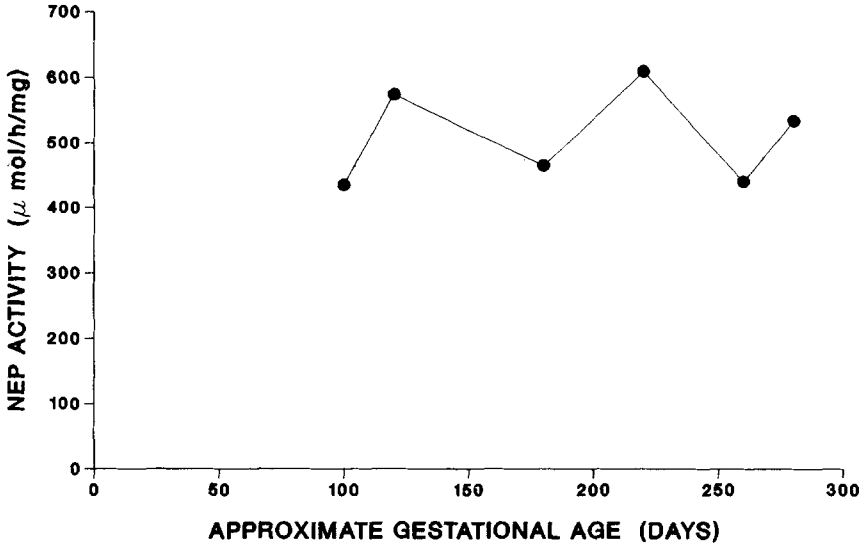

Fig. 2. NEP activity at different gestational ages. Ligaments were collected from fetuses at gestational ages ranging from 100 to 280 days, estimated by crown–rump measurement. The tissue was homogenized and a crude membrane fraction prepared as described in the text. NEP activity was measured using the dansylated substrate, DAGNPG; data are μM/h/mg protein, based on measurement of protein in the membrane fraction.

collagen fibers (C) and elastin (E) were obvious in ligament taken from late gestational stages (Fig. 3c). Cells in culture also formed a subcellular matrix containing collagen fibrils (Fig. 3d).

The cultures formed contact-inhibited monolayers of flattened cells with prominent nuclei. The cells stained uniformly with antibody to vimentin, an intermediate filament protein (Fig. 4a,b), but they did not stain with antibody to cytokeratin (not shown). Antibody to human renal NEP also recognized an antigen on fibroblasts from fetal calf ligament; the staining was punctate and concentrated in the perinuclear area (Fig. 4c,d).

Enzymatic Activity in Cultured Fibroblasts

Enzymatic activity was measured in fibroblasts cultured from the fetal calf ligaments and fetal calf lungs taken at early (100–200 day) and late (200–280 day) gestational ages. Figure 5 compares the specific activity of membrane preparations from ligament tissue, freshly isolated cells, cultured ligament cells, and cultured lung cells. Fibroblasts dissociated from the ligament by collagenase treatment had much less activity than did intact tissue, and cells cultured by outgrowth from ligament explants also had low activity compared with intact tissue. By contrast, fibroblasts cultured from fetal lungs had NEP activity equivalent to that in intact ligament tissue. The lung cells not only expressed high levels of NEP, but they retained activity through multiple passages in culture (data not shown).

Because the composition of the extracellular matrix can influence the expression of elastin by ligament fibroblasts [10], we wanted to determine whether a matrix synthesized by the more active lung fibroblasts could enhance NEP activity in the less active

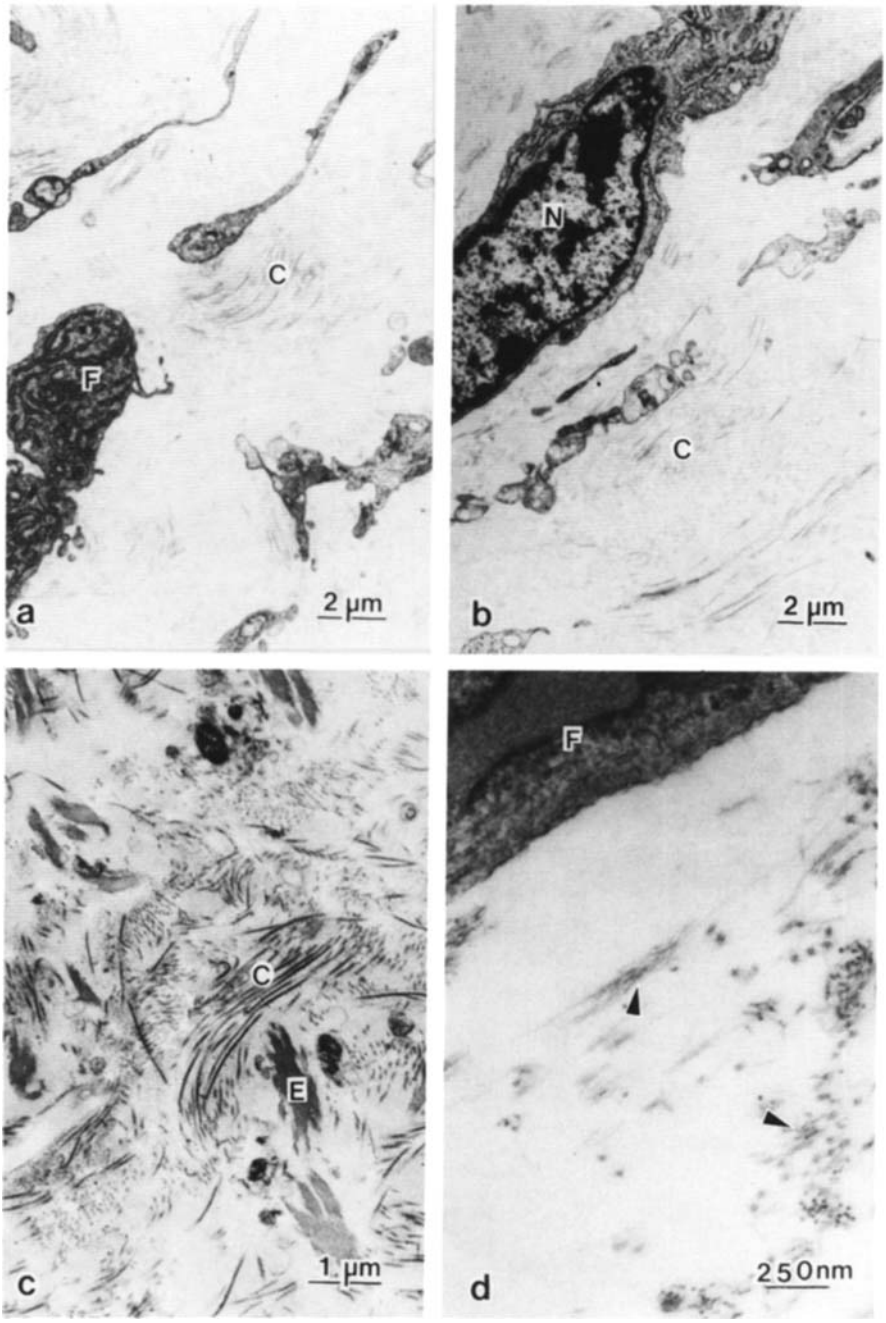

Fig. 3. Fibroblasts and matrix in nuchal ligament. **a,b:** Electron micrographs showing fibroblasts (F) with nucleus (N) embedded within a matrix containing collagen fibrils (C). **c:** Collagen fibrils and elastin (E) in a late gestational matrix after the cells were removed by repeated freezing and thawing. **d:** High magnification of fibroblasts (F) in culture and their extracellular matrix. Arrowheads indicate immature collagen fibrils.

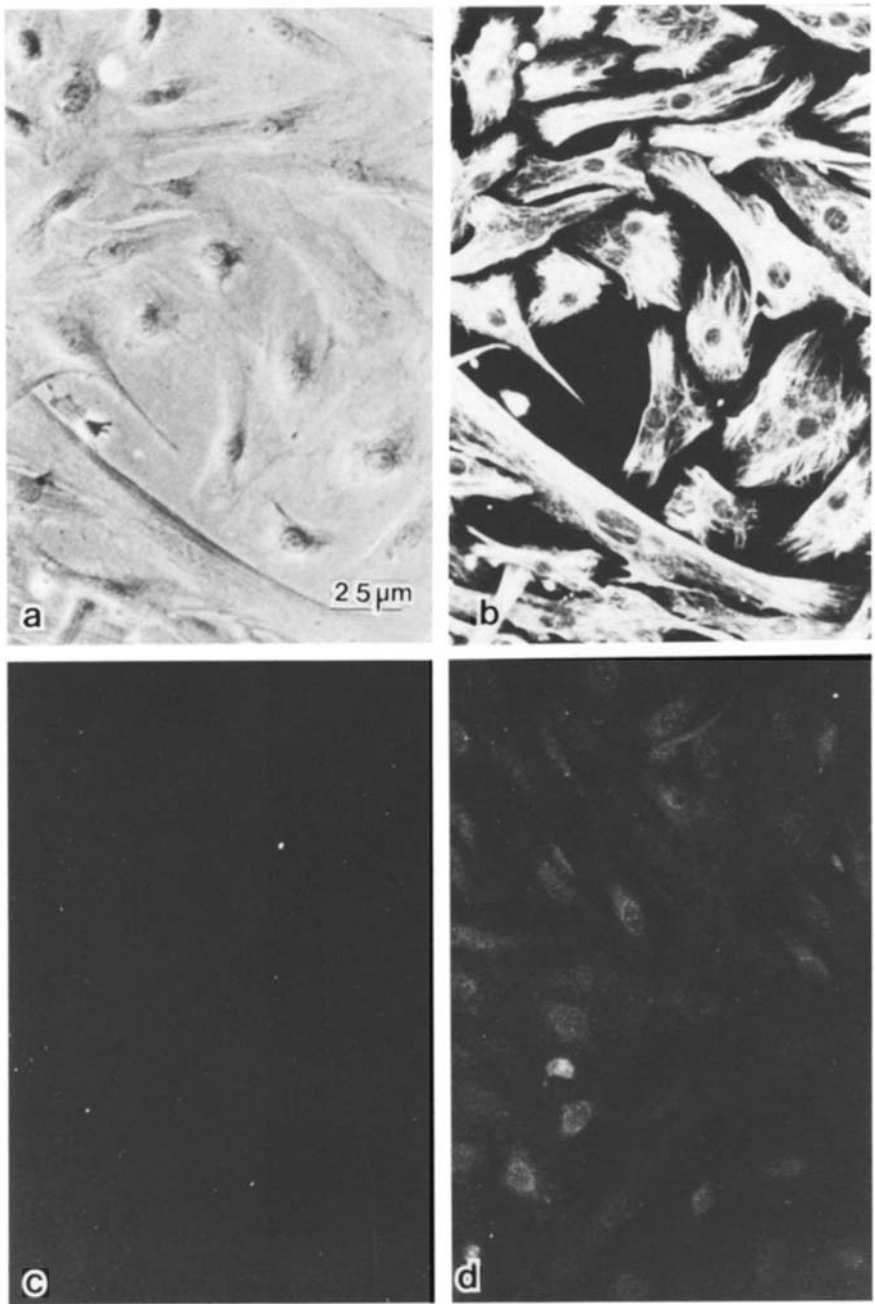

Fig. 4. Nuchal ligament fibroblasts in culture. Appearance of cells by phase contrast (**a**) and fluorescence (**b**) microscopy. **b:** Cells were treated with a primary antibody to vimentin followed by fluorescein-conjugated secondary antibody. Appearance of cells treated with normal rabbit serum (**c**) or rabbit antiserum to human renal NEP (**d**) developed with a fluorescinated antibody to rabbit IgG.

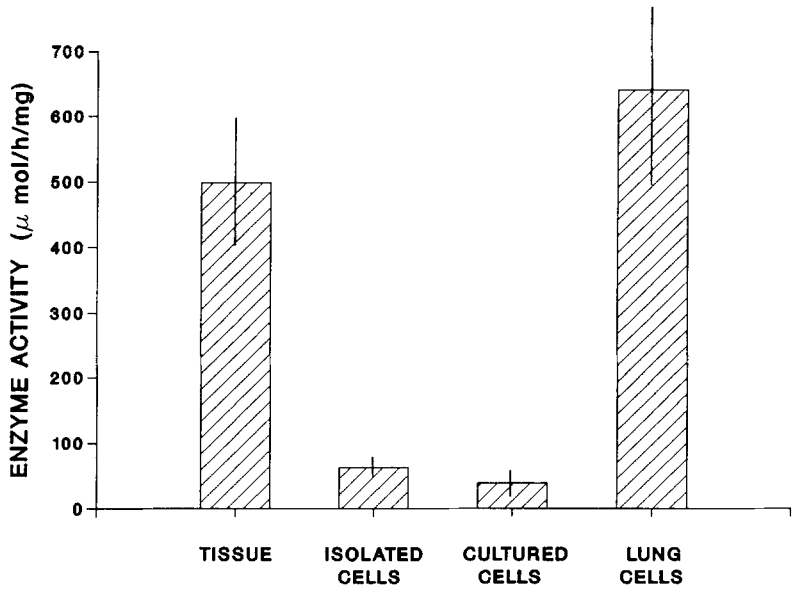

Fig. 5. Comparison of NEP activity in tissue, isolated cells, and cultured cells. Membrane preparations were prepared from tissue or cells and NEP was measured by hydrolysis of DAGNPG. The heights of the bars are means of enzymatic activity in three to five separate preparations; lines indicate SEM.

TABLE II. NEP Activity in Cultured Cells: Influence of Subcellular Matrix*

Cell type and treatment	NEP activity (μmol/h/10^6 cells)	% of control (plastic)
Experiment 1		
Plastic	50.2	100
Ligament matrix (240 day)	43.2	89
Lung matrix (280 day)	125.5	250
Experiment 2		
Plastic	20.0	100
Lung matrix (120 day)	33.8	169
Lung matrix (280 day)	80.0	400
Experiment 3		
Plastic	22.5	100
Lung matrix (120 day)	39.0	173
Lung matrix (280 day)	65.5	291

*Data are from three separate experiments in which cells were grown on preformed cell matrices or on plastic alone. Cells were cultured from ligament or lung tissue by collagenase digestion as described in the text. Ligament fibroblasts in Exp 1 were from tissue taken at 240 days of gestation, and those in Exp 2 and 3 were from tissue of 220 days gestation. The cells were plated on plastic or on a preformed matrix derived from fetal calf lung cells or ligament cells. Lung matrix was from cells cultured from either early (120-day) or late (280-day) gestational fetuses. Ligament matrix (Exp 1) was from late (240 day) gestational cells. When the monolayer of applied ligament cells was completely confluent (5–7 days) NEP activity was measured by cleavage of the dansylated substrate, DAGNPG as described in the text.

ligament fibroblasts. Table II shows that ligament fibroblasts grown on plates coated with extracellular matrix from lung fibroblasts had greater NEP activity than did those grown on plastic alone or on a matrix from ligament fibroblasts. The influence of an "older" matrix, derived from lung cells of a 280-day fetus, appeared to be slightly greater than matrix from younger (120-day) cells (Table II).

DISCUSSION

Neutral metallo-endopeptidases have been described in a variety of tissues. These enzymes have diverse roles, including inactivation of vasoactive peptides [1–4] and promotion of calcium-dependent membrane fusion in myoblasts [16,17] and neural synapses [18]. NEP (E.C. 3.2.24.11) is a 90- to 94-kD membrane-bound zinc metalloendopeptidase that is expressed by human fibroblasts [5,6]. Small peptides appear to be the preferred substrates for NEP, and the cell-surface localization of this enzyme is ideally suited for regulation of peptide signals [1].

This study confirms that the metallo-endopeptidase in nuchal ligament of fetal calves is both enzymatically and immunologically similar to the neutral endopeptidase from human kidney. Membrane fractions from either tissue displayed NEP-like activity, as determined by cleavage of the dansylated substrate, DAPGN, and both ligament and renal enzymes were more than 90% inhibited by phosphoramidon, an inhibitor of NEP. Like the kidney preparation, the specific activity of the ligament enzyme increased approximately 10-fold following separation by FPLC. Both enzymes eluted at the same salt concentration.

The ligament enzyme was recognized by a polyclonal antibody to purified human renal neutral endopeptidase. Western blotting showed that both calf ligament and human kidney enzymes migrate in polyacrylamide gels with a molecular weight of ~92 kD. Taken together with the biochemical data, these observations indicate that the enzyme in fetal ligament is similar to NEP from human kidney [13] and placenta [14]. Comparison of sequences in enzyme cloned from rabbit, rat, or human sources [19,20] indicates a high degree of conservation among mammalian species.

The ligamentum nuchae in early fetal life contains a fibroblastic mesenchymal cell that undergoes morphologic and biochemical changes during development [21–23]. This fibroblastic cell is the most likely source of NEP. Elastin synthesis within the ligament begins at ~180 days of fetal life [23,24], and pre-elastogenic fibroblasts produce elastin when grown on extracellular matrices from late gestational cells, suggesting that the timing of this event is dictated by inductive interactions between the cells and their secreted extracellular matrix [10].

Sections of nuchal ligament examined by electron microscopy showed a homogeneous population of cells embedded within a connective tissue matrix. The matrix contained collagen fibrils and, in tissue from late gestational samples, there were deposits of elastin. The location of an NEP-like enzyme in the fetal ligament [9] and its ability to hydrolyze a variety of small peptide substrates led us to speculate that, like elastin, the expression of NEP might change during fetal development. Our data on NEP activity in ligaments taken at various gestational ages indicate, however, that this enzyme is expressed within the first trimester of fetal life and persists through the remaining period of gestation.

NEP activity in nuchal ligament fibroblasts may depend upon the development and maintenance of an appropriate substratum. Cells dissociated from ligament tissue

by collagenase had greatly reduced activity compared to the intact tissue, even after several passages in culture, and reaction with an antibody to human renal NEP was only faintly positive. Proteolysis by contaminants in the collagenase used to dissociate the cells from ligament tissue might destroy surface membrane proteins. However, ligament fibroblasts that were derived from tissue explants, where no proteolytic enzymes are used, also had low activity, and electron micrographs of the cultured cells showed that they produce a collagen-containing matrix. By contrast, lung fibroblasts, derived either from explants or collagenase digestion, had levels of NEP that were comparable to those in intact ligament tissue. These cells also retained NEP activity through multiple passage in culture.

Further support for the idea that NEP expression in cultured ligament fibroblasts depends, in part, on contact with specific elements within a subcellular matrix is offered by experiments in which ligament fibroblasts were grown on matrices of lung fibroblasts. Cells in contact with a lung cell matrix had higher enzymatic activity than did cells cultured on their own matrix or on plastic. Although we have not yet identified and analyzed the potential differences between extracellular matrices from lung and ligament fibroblasts, our data suggest that matrix composition influences NEP activity.

Elastin and elastin degradation products are potentially important for phenotypic modification of fibroblasts. Elastin peptides were shown to be chemotactic for fibroblasts [25], and a protein on the surface of mesenchymal cells (elastonectin) was shown to mediate cellular adhesion to insoluble elastin [26]. Differences in elastin content of the matrix, however, would not likely account for differences in NEP expression because the enzyme is present at early (100–120 days) gestational ages, and elastin synthesis begins only after 180 days [23,24]. Furthermore, a subcellular matrix from late gestational (220 days) ligament fibroblasts had no effect on NEP activity. Although we do not yet have evidence to indicate which component(s) of the extracellular matrix might influence the activity of NEP within the nuchal ligament, the persistence of the enzyme throughout a range of fetal ages and the fact that activity is influenced by the extracellular environment suggest that NEP may be involved in growth and development of the fetal bovine nuchal ligament.

ACKNOWLEDGMENTS

This work was supported by grants from NIHLBI, HL36545, HL36538, and HL39943 from the National Institutes of Health. We thank Ms. Cassandra McArthur for assistance with gel electorphoresis and immunoblotting of the enzymes and Ms. Patricia Genoway for assistance with computer-generated illustrations. We thank Dr. Ervin G. Erdös of the University of Illinois at Chicago for the antibody to human renal neutral endopeptidase.

REFERENCES

1. Erdös EG, Skidgel RA: FASEB J 3:145–151, 1989.
2. Connelly JC, Skidgel RA, Schulz WW, Johnson AR, Erdös EG: Proc Natl Acad Sci USA 82:8737–8741, 1985.
3. Painter RG, Dukes R, Sullivan J, Carter R, Erdös EG, Johnson AR: J Biol Chem 263:9456–9461, 1988.
4. Yuli I, Snyderman R: J Biol Chem 261:4902–4908, 1986.
5. Johnson AR, Ashton J, Schulz WW, Erdös EG: Am Rev Respir Dis 132:564–568, 1985.

6. Lorkowski G, Zijderhand-Bleekemolen JE, Erdös EG, Von Figura K, Hasilk A: Biochem J 248:345–350, 1987.
7. Letarte M, Vera S, Tran R, Addis JBL, Onizuka RJ, Quackenbush EJ, Jongneel CV, McInnes RR: J Exp Med 168:1247–1253, 1988.
8. Shipp MA, Vijayaraghavan J, Schmidt EV, Masteller EL, D'Adamio L, Hersch LB, Reinherz EL: Proc Natl Acad Sci USA 86:297–301, 1989.
9. Sullivan J, Johnson AR: Biochem Biophys Res Commun 162:300–307, 1989.
10. Mecham RP, Senior RM: In Reddi AH (ed): "Extracellular Matrix: Structure and Function." New York: Alan R Liss, Inc, 1985, pp 383–392.
11. Bradford MM: Anal Biochem 72:248–254, 1976.
12. Florentin D, Sassi A, Roques BP: Anal Biochem 141:62–69, 1984.
13. Gafford J, Skidgel RA, Erdös EG, Hersh LB: Biochemistry 22:3265–3271, 1983.
14. Johnson AR, Skidgel RA, Gafford JT, Erdös EG: Peptides 5:789–796, 1984.
15. Helfman DM, Hughes SH: Methods Enzymol 52:451–469, 1987.
16. Couch CB, Strittmatter WJ: Cell 32:257–265, 1983.
17. Lassar AB, Paterson BM, Weintraub H: Cell 47:649–656, 1986.
18. Schwartz JC, Malfroy B, De La Blume S: Life Sci 29:1715–1740, 1981.
19. Devault A, Lazure C, Nault C, Le Moual H, Seidah NG, Chretien M, Kahn P, Powell J, Mallet J, Beaumont A, Roques BP, Crine P, Boileau G: EMBO J 6:1317–1322, 1987.
20. Malfroy B, Schofield PR, Kuang W-J, Seeburg PH, Mason AJ, Henzel WJ: Biochem Biophys Res Commun 144:59–66, 1987.
21. Mecham RP, Lange G, Madaras J, Starcher B: J Cell Biol 90:332–338, 1981.
22. Jones CJP, Sear CHJ, Grant ME: J Pathol 131:35–53, 1980.
23. Davidson JM, Smith K, Shibahara S, Tolstoshev P, Crystal RG: J Biol Chem 257:747–754, 1982.
24. Mecham RP, Madaras JG, Senior RM: J Cell Biol 98:1804–1812, 1984.
25. Senior RM, Griffin GL, Mecham RP: J Clin Invest 70:614–618, 1982.
26. Hornbeck W, Tixier JM, Robert L: Proc Natl Acad Sci USA 83:5517–5520, 1986.