Cell Adhesion and Communication: MANUSCRIPT 004/94R
Compositor: Bucks County Type & Design BCT&D Job #7149

Cell Adhesion and Communication, 1995, Vol. 3, pp. 0–0
Reprints available directly from the publisher

Growth Stimulation of Human Skin Fibroblasts by Elastin-Derived Peptides

A. KAMOUN[1], J.-M. LANDEAU[1], G. GODEAU[1], J. WALLACH[2], A. DUCHESNAY[1], B. PELLAT[1]* and W. HORNEBECK[1]*

[1]*Biochemistry department, Faculty of Dental Surgery, University René Descartes PARIS V, 1 rue Maurice Arnoux, 92120 Montrouge, France;* [2]*Laboratoire de Biochimie analytique et synthèse bioorganique, University Claude Bernard LYON I, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France*

Received March 26, 1995

Elastin-derived peptides (kappa-elastin : κE, mean molecular mass : 75 kDa), either coated onto plastic dishes or added to culture media (0.26 to 1.33 nM) stimulated the growth of human skin fibroblasts (HSF) strains obtained from different donors and tested at different cell passages (4 to 12). Coated 44.4 $\mu g/cm^2$ insoluble elastin (iE) exhibited the same action; coated iE or κE significantly modifies the HSF morphology: after 5-6 days of culture, HSF are more elongated, and at preconfluence state, formation of HSF clusters surrounding iE were observed. Increased ^3H thymidine incorporation and proliferative effect of HSF by κE (1.3 to 2.2 fold as compared to control cells) was observed after a lag phase period which raised with initial HSF density. Optimal proliferative effect was obtained at κE 8.5 10^{-10} M, a value close to the dissociation constant (k_D = 2.7 10^{-10} M) of κE to HSF. Valine-glycine-valine-alanine-proline-glycine (VGVAPG), but not valine-glycine-valine (VGV) or Valine-glycine-valine-valine-glycine-alanine (VGVVGA) also significantly stimulated, optimally at 7.0 10^{-10} M, HSF proliferation. It was concluded that the stimulatory influence of elastin derived peptides on HSF proliferation was mediated through a binding to plasmalemmal receptor of HSF.

KEYWORDS: fibroblasts, elastin, elastin-derived peptides

ABBREVIATIONS: human skin fibroblasts = HSF; fetal calf serum = FCS; smooth muscle cells = SMC; pseudoxanthoma elasticum = PXE; κ-elastin = κE; insoluble elastin = iE; valine-glycine-valine = VGV; valine-glycine-valine-alanine-proline-glycine = VGVAPG; valine-glycine-valine-valine-glycine-alanine = VGVVGA.

INTRODUCTION

Extracellular matrix macromolecules interact with proteins of the plasmic membrane (Ruoslahti and Pierschbacher, 1987; Liotta et al., 1986). These interactions trigger some cellular responses involved in the tissue remodeling (Boucaut et al., 1984; Werb et al., 1989). Earlier investigations indicated that the binding of tropoelastin and elastin-derived-peptides to fibroblasts was via a receptor (Mecham et al., 1989; Groult et al., 1991). An hexapeptide sequence several times repeated in tropoelastin sequence, e.g. Valine-glycine-valine-alanine-proline-glycine (VGVAPG) defines the receptor binding site on the molecule (Mecham et al., 1989); on human skin fibroblasts, the elastin receptor is a 67 kDa glycoprotein with lectin-like properties (Groult et al., 1991), related to an enzymatically inactive, alternatively spliced form of β-galactosidase (Hinek et al., 1993). It was demonstrated that this receptor was coupled with phospholipase C through a pertussis-toxin sensitive GTP binding G protein; its occupancy by elastin derived peptides was also accompanied by the hydrolysis of phosphoinositides by phospholipase

Address correspondence to: B. Pellat or W. Hornebeck , Biochemistry Department, Faculty of Dental Surgery, University René Descartes PARIS V, 1 rue Maurice Arnoux, 92120 Montrouge, France. Tel.: (1) 46 57 12 86, Fax: (1) 42 53 43 25.

C, a property common to growth factors and some agonists (Jacob and Hornebeck, 1985).

In this study, we demonstrated that elastin derived peptides (κE) stimulated the proliferation of human skin fibroblasts (HSF). Maximal growth stimulatory effect of κE occured at concentrations approximating the k_D of binding between κE and fibroblasts(Groult et al., 1991). Since such HSF growth promoting activity could be also obtained with VGVAPG, it was assumed that interactions between elastin and its receptor is responsible of the HSF proliferation.

MATERIALS AND METHODS

Biochemical Agents

Insoluble elastin (iE) was purified from calf ligamentum nuchae using the hot alkali procedure (Jacob and Hornebeck, 1985). Its purity was checked by amino acid analysis and lack of hexoses and hexosamines. Elastin-derived-peptides were obtained following 1 M KOH / 80% ethanol hydrolysis of insoluble ligamentum nuchae elastin (Jacob and Hornebeck, 1985). The hydrolysate was allowed to coacervate; the coacervate was dissolved in 0.5 M Tris/HCl, 1 M NaCl, pH 7.5 and fractionated by gel permeation on Sephadex G100; a major fraction (Mr 75,000) was isolated for this investigation. It presents a composition of amino acids similar to its insoluble counterpart, with a slightly lower Gly/Ala ratio (Jacob and Hornebeck, 1985). Fibroblast growth factor 2 (FGF-2) was a generous gift of Dr Josette Badet (URA 1813 CNRS, Créteil, France).

Peptide Synthesis

Valine-glycine-valine (VGV), valine-glycine-valine-alanine-proline-glycine (VGVAPG) and valine-glycine-valine-valine-glycine-alanine (VGVVGA) were synthesized by the solid-phase method with N-FMOC L-amino acids. The first aminoacid (Ala, Gly or Val) was coupled as FMOC-aminoacid-pentafluorophenylester (Pfp) (5 M excess). After the linkage of the C-terminal residue, all FMOC-aminoacids were coupled using 3.5 molar excess in presence of 1-hydroxybenzotriazole with a coupling time of 60 minutes. Each Fmoc deprotection time involved treatment with 20% piperidine/dimethylformamide for 10 minutes. Cleavage of the peptide was achieved by a 6 hours treatment with trifluoracetic acid-water (95/5, v/v), followed by successive washings of the resin with ether. Purity of the peptides was checked by HPLC and their molecular weight confirmed by fast atom bombardment mass spectrometry.

Human Skin Fibroblasts (HSF) Cultures

HSF were obtained from outgrowth of explants obtained after breast plastic surgery (18, 32 and 38 year old women), earskin plastic surgery (23 year old woman), from skin biopsies of a 25 year old patient suffering from pseudoxanthoma elasticum (PXE) and another patient (18 year old) with homocystinuria (with their informed consent). Cells were seeded at 1.2-1.6 10^4 density in 1.7 cm diameter dishes (Costar®; Brumath, France, 24 wells-culture plates) and cultured in Dulbecco Modified Eagle's medium (DMEM; Gibco®; Paisley, Scotland) supplemented with 10 per cent fetal calf serum containing 200 U of penicillin and 200 µg/ml streptomycin at 37°C in a 95% air, 5% CO_2 atmosphere. The cell media were changed every 48 hours, with or without elastin derived peptides. Growth parameters were studied at times: i) following seeding and cell attachment, ii) at 2-4 days, iii) at preconfluent state, between days 5 and 6, according to the fibroblast strain. All the experiments were performed with cells between the 3rd and the 12th passage. Smooth muscle cells were obtained from thoracic aorta explants of a 3 weeks-old minipig.

Fibroblasts Proliferation Measurements

Medium was removed and cells were harvested by 0.05% trypsin in calcium-free PBS. The viability of cells was determined on an hemocytometer by trypan blue exclusion. ^3H thymidine (10.36 x 10^{11} Bq/mmol, Amersham, France) incorporation into cell DNA was determined at different times of cell cultures. Briefly, cell wells were seeded with 1 to 3 10^4 fibroblasts (five wells in each experiment) with DMEM containing 10% fetal calf serum (FCS). Cell wells were coated with elastin derived peptides; in some experiments, peptides were added directly to HSF culture media. At set time of cell growth, from 2 hours (the time required for fibroblast attachment), to 6 days, cell media were withdrawn, fibroblasts were rinsed with PBS, and the cultures were synchronized for 24 hours in DMEM lacking 10% fetal calf serum but supplemented with 0.1% bovine serum albumin. Culture media were eliminated and

the cells washed twice with DMEM. Then ^{3}H thymidine (3.7×10^4 Bq per dish) was added for 4 hours at 37 °C. DNA was precipitated with 0.5 ml of 10% trichloracetic acid after 15 min. at 4°C, the precipitates were rinsed 3 times with 0.5 ml of distilled water and finally the pellets were dissolved in 0.3 M NaOH. Aliquots were taken for counting.

Coating of Culture Dishes

κE was sterilized by filtration and iE by autoclaving. One hundred µl of these preparations were placed into 1.7 cm diameter micro wells (Costar^R, Brumath, France), and the corresponding plates were stirred overnight at 25°C under UV light to allow the solvent to evaporate; they were then rinsed twice with PBS and twice with DMEM.

Cell Morphometric Studies

Fibroblasts grown on different supports were fixed with 70% alcohol and stained with toluidine blue. The mean shape factor (SF) of the cells was defined as: "SF = L x l / L + l", L and l representing respectively the length and maximal width of the cell (Rasoamanantena et al., 1993). SF values were automatically determined using a specific program (Bertaux et al., 1991).

Statistical Analysis

The statistical significance of the variations observed were analyzed using the Student-Fisher t-test.

RESULTS

1.4×10^4 HSF (from a 23 year old woman, 3rd passage) were first allowed to attach and spread onto plastic dishes either uncoated or coated with 100 µg κE or iE (44.4 µg/cm^2). No significant differences in the extent of HSF attachment were observed between κE, iE and the plastic support. Also no significant differences in HSF shape and density could be visualized at day 2 of culture under our experimental conditions. After 4 days however, a marked increase in cellular density could be observed when fibroblasts were cultured onto κE or iE (Figure 1, a, b, c). Such an effect was even more pronounced after 8 days of culture and at this stage, the formation of fibroblast clusters surrounding iE was noticed (Figure 1 f). Cell clusters were also observed at day 4 for fibroblasts cultured onto κE. Fibroblasts grown onto κE and/or iE appeared to exhibit a significantly lower shape factor (SF), an indication of their more elongated shape (Figure 1).

κE and iE influences on HSF proliferation were reproducible using cells from different donors: 4 healthy ones and one PXE patient. κE growth promoting activity, after 8 days of culture, ranged from 28 to 92% increase as compared to controls (fibroblasts cultured onto plastic). iE coating resulted in similar enhancement of fibroblasts proliferation (Table 1). A decreased influence of κE or iE on fibroblasts growth was observed with cell passages and no effect was demonstrated on fibroblasts obtained from one patient suffering from homocystinuria (Table 1). iE possessed a greater influence on porcine aorta smooth muscle cells (SMC) proliferation than κE: 70% increase for κE as compared with 145% increase for iE.

For all strains analyzed, a lag phase period of 3-4 days was observed before any enhancement of HSF proliferation induced by κE coating could be statistically demonstrated. This is illustrated on Figure 2. After 3 days of culture, rates of proliferation were similar whatever HSF were cultured on plastic support ($2.14 \pm 0.2\ 10^4$) or κE ($2.28 \pm 0.27\ 10^4$). However, HSF had proliferated more rapidly when grown on κE for 5 days, and this effect was even more pronounced after 8 days of culture (Figure 2). At this time, the number of HSF was respectively equal to $2.0\ (\pm\ 0.3)\ 10^5$ on κE and $1.0\ (\pm\ 0.2)\ 10^5$ on plastic dishes. The proliferating effect of κE on fibroblasts growth, as well as the delay time of this phenomenon, was confirmed by ^{3}H thymidine incorporation into cell DNA. After 2 or 3 days of culture, no difference in ^{3}H thymidine uptake was observed between cells cultured on plastic or κE; at day 5, there was a two fold increase of ^{3}H thymidine incorporation when cells were grown onto a κE support (Figure 2, inset).

To exclude inaccuracy of κE concentrations by the coating technique, κE (100 µg : 1.33 nmole) was added at the beginning of the cultures and HSF were seeded at different densities (1 to $3\ 10^4$ cells). Whatever HSF seeding density, κE exerted its growth promoting activity; it needs to be emphasized however, that enhancement of HSF proliferation induced by κE was significant earlier (after 2-3 days of culture) when cells are seeded at the highest density (Figure 3).

Then, the influence of κE concentration on HSF

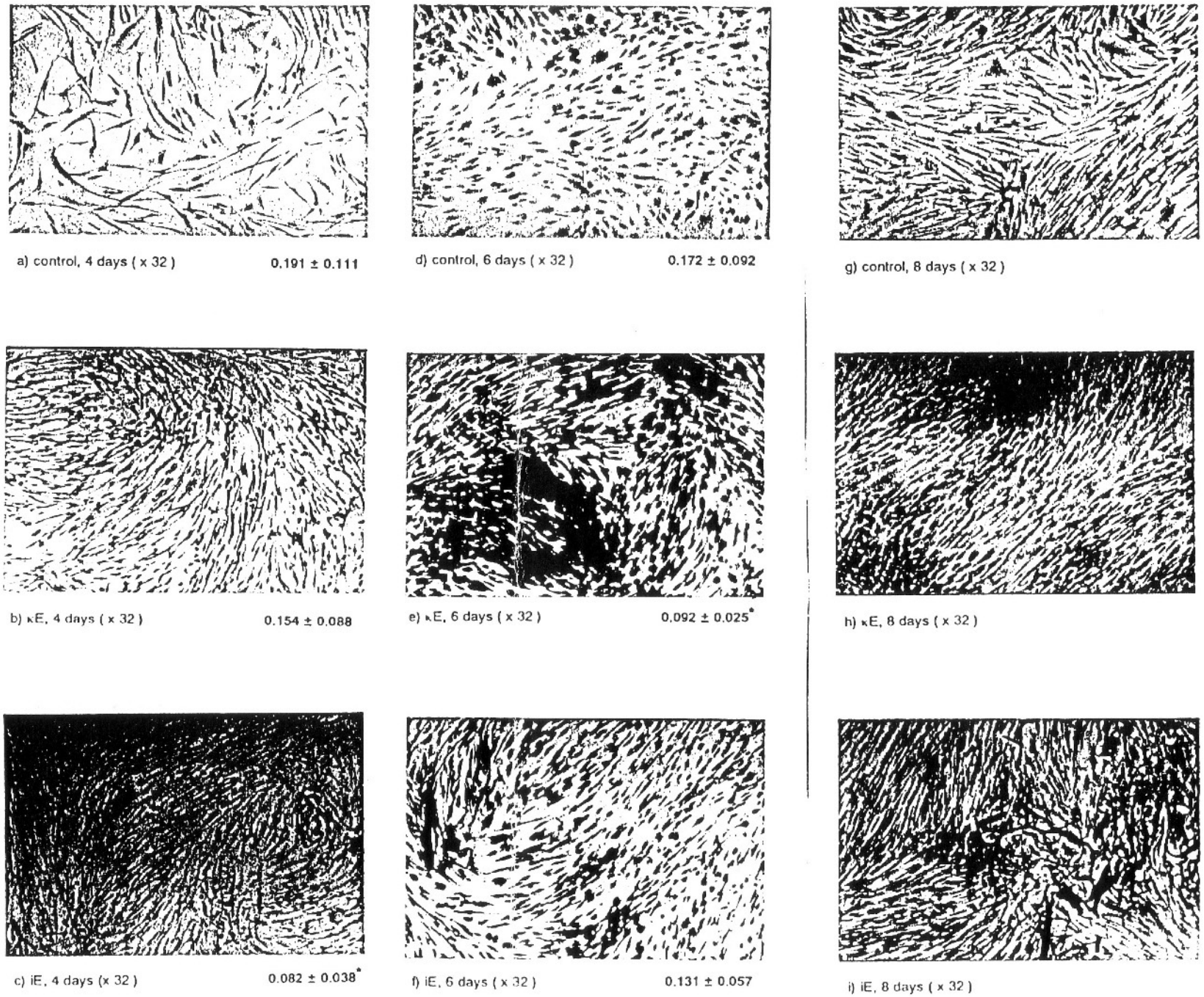

FIGURE 1. Effect of coated elastin-derived peptides (κE) or insoluble elastin (iE) on morphology of human skin fibroblasts. Fibroblasts were cultured on plastic dishes without or with 100 µg coated peptides; 1.4 ± 0.2 x 10^4 cells (from a 23 year old patient) were added to each well and the cultures were performed in DMEM DMEM supplemented with 10% fetal calf serum. In figure a to f, the cell shape factor is in the lower right corner as mean ± SD.

TABLE 1.
Effect of Coated κE or iE (100 μg Coating) on the Proliferation of Fibroblasts

Cell origin	Age of patient (years)	Passage numbers	Initial cells density (x 10^{-4})	Cell counts (8 days of culture) x (10-4) Controls	coated κE (100 μg)	coated iE (100 μg)
A - Fibroblasts from breast plastic surgery healthy individuals						
1	18	8	1.4	15.3 ± 1.15	23.5 ± 0.3*	21.9 ± 0.78*
2	38	5	1.3	20.70 ± 1.86	36.00 ± 2.83	39.00 ± 5.29
3	32	4	1.4	21.00 ± 3.16	33.80 ± 2.36*	34.5 ± 2.65*
4	32	10	1.6	6.30 ± 0.49	8.06 ± 0.52*	8.31 ± 0.60*
B - Skin biopsies						
1 - Patient PXE	28	10	1.6	3.38 ± 0.49	4.15 ± 0.24*	4.24 ± 0.36*
2 - Patient HC	18	5	1.1	4.80 ± 0.16	4.27 ± 0.37	4.78 ± 0.60
C - Porcine (3 week old minipig) aorta smooth muscle cells		5	1.6	10.64 ± 1.51	18.10 ± 2.1*	26.00 ± 1.53*

PXE = pseudoxanthoma elasticum
HC = homocystinuria
* : $p < 0.01$) as compared with control cells.

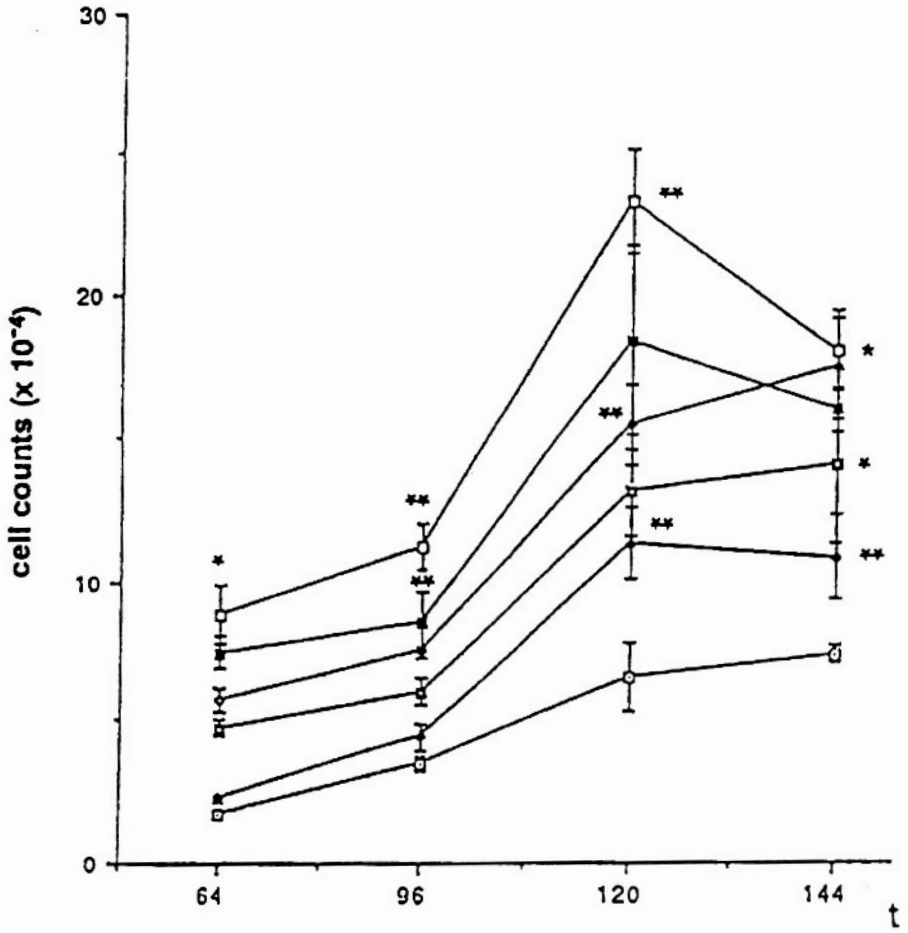

FIGURE 2. Effect of coated elastin-derived peptides (κE) on the proliferation of fibroblasts. Fibroblasts were cultured on plastic dishes without (—o—) or with (—•—) κE 44.4 μg/cm².
** indicates a significant difference ($p < 0.01$) observed for the same time of culture; results are the mean ± SEM of experiments for the cellular counts and for 5 experiments for ^{3}H-thymidine incorporation in DNA (mean ± SEM).

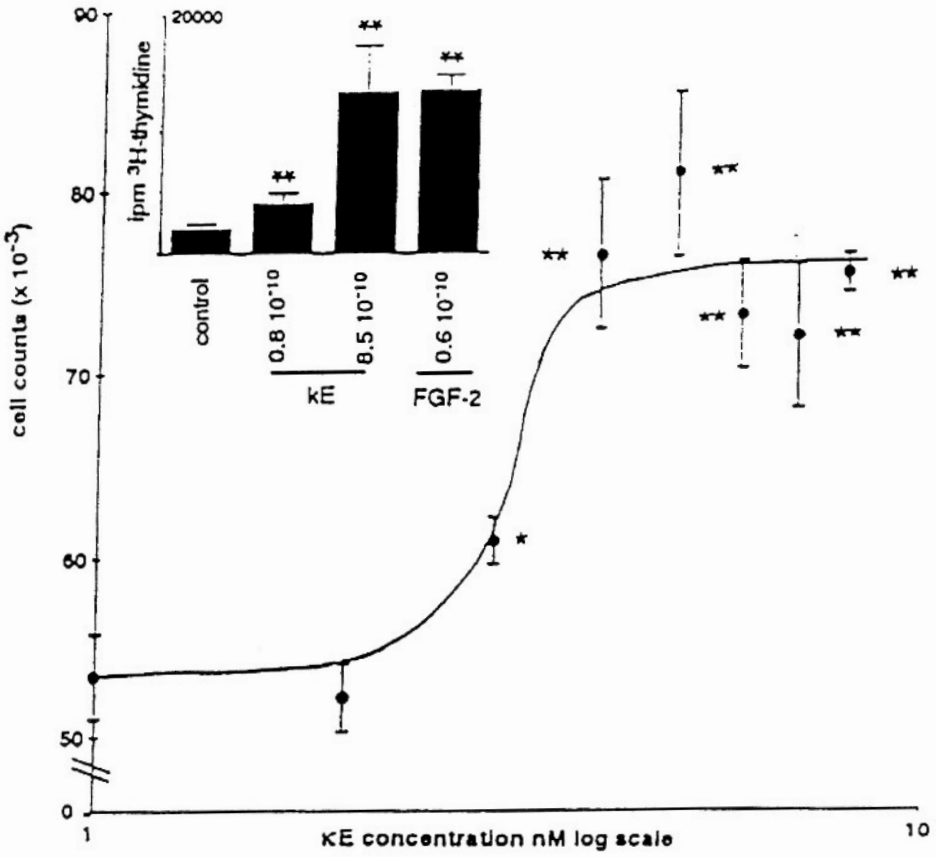

FIGURE 3. Effect of elastin-derived peptides (κE) on the fibroblasts proliferation. Fibroblasts from a 23 year old subjectwere used with or wothout κE (1.3 nM) in DMEM containing 10% FCS. Fibroblasts were at the 7th passage and used at an initial density of 1.0, 2.0 or 3.0 x 10^4 cells / microwell. Results were determined after 6 days of culture. Statistical significance between cultures with or without κE :
* : $p < 0.05$
** : $p < 0.01$
Initial density of cells :
1 x 10^4 HSF Control
2 x 10^4 HSF Control
3 x 10^4 HSF Control
1 x 10^4 HSF kE
2 x 10^4 HSF kE
3 x 10^4 HSF kE

proliferation was studied; in keeping with above data, ^{3}H thymidine incorporation was quantified in synchronized cells (seeded at 1 10^4 cells / dish) at day 4 of HSF culture. Radioactivity inorporation increased with κE concentration and was followed 24 hours later by a about two-fold enhancement of cell counts. Maximal effect was attained for κE concentration equal to 8.5 10^{-10} M; it was equivalent to the HSF growth promoting activity of a 0.6 10^{-10} M concentration of FGF-2 (Figure 4).

To investigate whether κE effect on HSF growth was mediated through binding of elastin-derived peptides to its receptor, similar experiments were performed using synthetic peptides: VGVAPG, the hexapeptidic sequence which interacts with the 67 kDa elastin receptor (Mecham et al., 1989; Groult et al., 1991), VGV tripeptide and VGVVGA, another hexapeptidic sequence not found in bovine or human tropoelastin sequences. Similarly as with κE experiments, HSF were cultured for 5 days in DMEM supplemented with 10% fetal calf serum; at day 5, the cells were cultivated in serum free DMEM containing increasing concentration of peptides. The statistically significant enhancing influence of VGVAPG on HSF growth was demonstrated (Figure 4). Maximal effect was observed with 7 x 10^{-10} M VGVAPG, which is close to values obtained with κE. In contrast, VGV and VGVVGA did not reveal significant growth promoting activities.

DISCUSSION

Previous studies demonstrated that elastin peptides may bind to a cell membrane receptor coupled to a G-protein and phospholipase C (Groult et al., 1991;

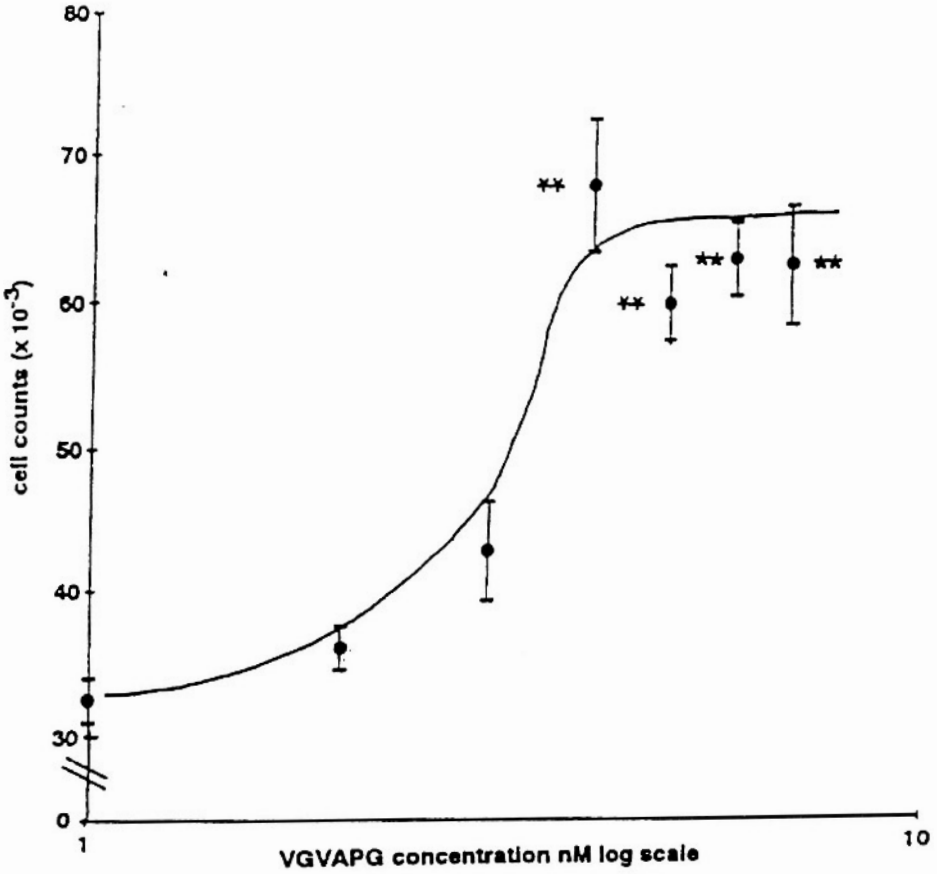

FIGURE 4. Dose-effect relationship for the proliferative action of elastin-derived peptides (κE). Fibroblasts were used as in figure 4 with initial density of 1.5×10^4 cells / microwell and κE was added for 24 hours after an initial culture of 5 days. From a 23 year old individual, 7th passage were seeded at and grown for 5 days in DMEM containing 10% FCS, then maintained for 24 hours in DMEM containing increased amounts of κE.
Insert: after 5 days of culture, fibroblasts were synchronized for 24 hours and ^3H thymidine incorporation was determined as described in Methods.
* : $p < 0.05$
** : $p < 0.01$.

Varga et al., 1989; Ghuysen-Itard et al., 1993). Activation of this 67 kDa elastin receptor led to an increase formation of inositol-tri-phosphate (IP_3) and also opened receptor-dependent calcium channels Varga et al., 1989).

We showed here, that κE, either coated onto culture wells, or directly added to cell culture media could promote growth of several human fibroblasts strains. Under our experimental conditions using low κE amounts (<100 µg), a lag phase of 3 to 5 days was observed before any significant proliferative action of κE could be demonstrated. It was recently reported however, that higher κE levels (3 to 5 mg/ml) could stimulate chinese hamster lung fibroblasts proliferation in the first 24 hours of culture (Ghuysen-Itard et al., 1992). The reason for such a delay in κE proliferative action is unknown. Since κE effect on HSF proliferation varied with cell seeding density, it could be assumed that its effect might be partly dependent on cellular factors expressed by confluent cells and not by sparse cells; alternatively, it could correspond to a differential expression or mobilisation of elastin receptor at different stages of cell proliferation and/or in vitro aging (Hornebeck et al., 1986).

Proliferation effect of elastin on HSF was accompanied, at the late stages of cell growth i.e. near to cell confluency, by a change in fibroblasts shape which took a more elongated appearance and in some areas accumulate around elastin fibers. Such close contact between iE and fibroblasts also induced cytosketon microfilaments reorganization (Perdomo et al., 1994). HSF proliferation was observed in all HSF from healthy donors we have analyzed; a trend to a decreased effect, in terms of intensity, was noticed with cell passages. It has to be emphasized that elastin biosynthesis was also reported to slow down with in vitro HSF aging (Davidson et al., 1990). Fibroblasts obtained from one PXE patient, an hereditary disease characterized by elastin accumulation in the dermis of patients, associated with major morphological modifications of elastic fibers (PXE) (Uitto et al., 1989), also exhibited a higher proliferating rate when cultured in presence of iE or/and κE; in contrast, growth promoting activity of κE as well as iE was not observed for HSF from one patient with homocystinuria, a disease also characterized by elastic fibers abnormalities (Myers et al., 1985). Other cell types like chinese hamster lung fibroblasts (Ghuysen-Itard et

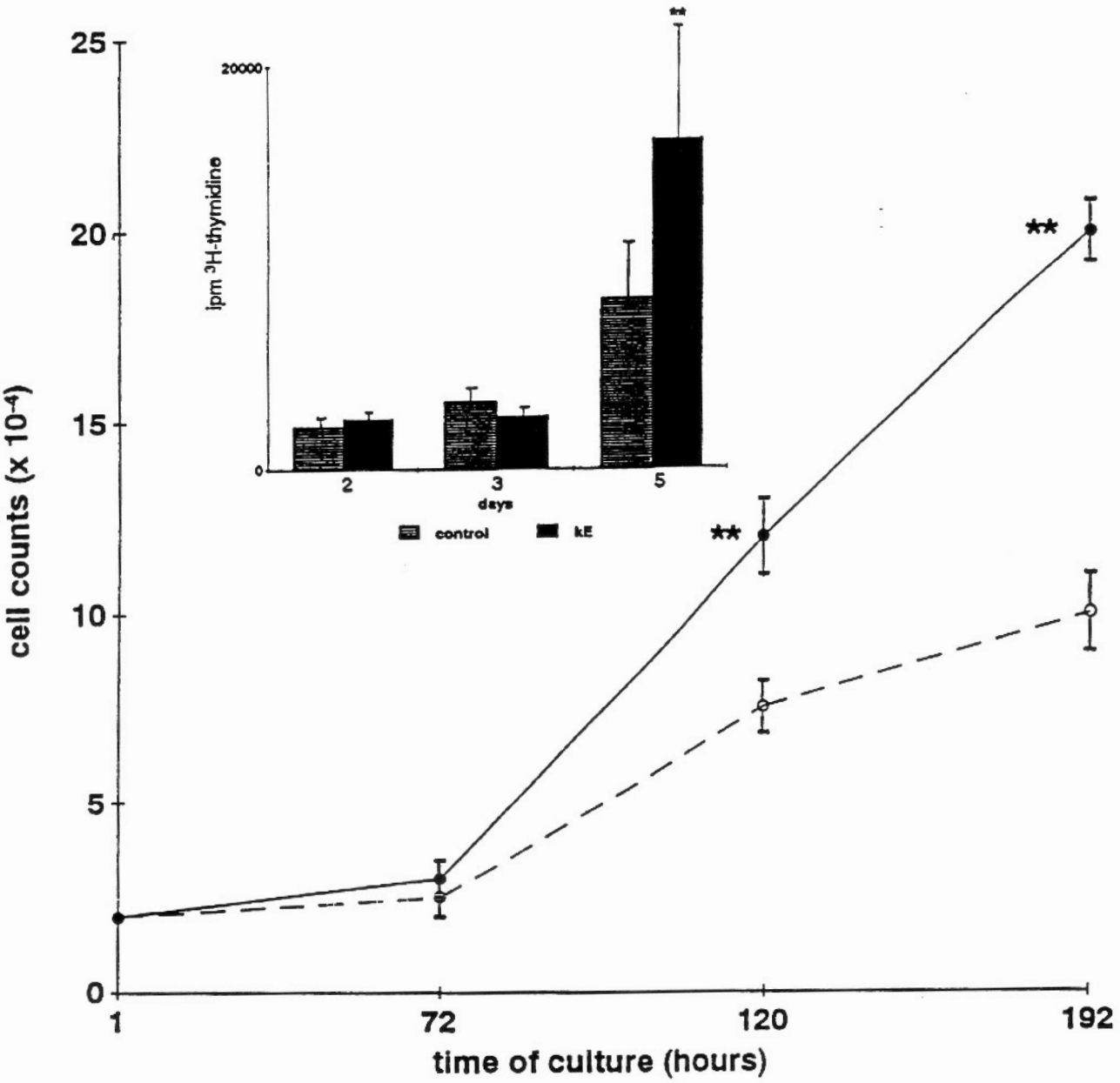

FIGURE 5. Dose-effect relationship for the proliferative action of Val-Gly-Val-Ala-Pro-Gly (VGVAPG). Experimental conditions were identical to those of Figure 4.

al., 1992) and porcine aorta smooth muscle cells also responded to κE peptides; in contrast, either rat aorta SMC or rat skin fibroblasts were poor responders [not shown]; it should be pointed out that the VGVAPG sequence recognizing the elastin receptor is not present in rat tropoelastin (Sandberg et al., 1990). A pretreatment of cells by a molar excess vs κE of such hexapeptide but not a truncated one, VGV does inhibit κE but not fibronectin growth effect for HSF (not shown). These data suggested that κE proliferative effect, as other biological activities exerted by elastin derived peptides, was mediated by interactions of κE with its cell surface receptor. Indeed, VGVAPG, an hexapeptide sequence repeated 3 and 6 times respectively in human and calf tropoelastin (Yeh et al., 1987), exhibited growth factor activity for HSF. Again, optimal effect of hexapeptide on cell growth was obtained at concentrations similar to those inducing for example, chemotactic effect on several cellular types which express the 67 kDa elastin receptor (Senior et al., 1984; Homsy et al., 1988).

In vivo, elastin derived peptides could be generated by the action of neutral elastase-type protein-

ases which expression is exacerbated by inflammatory stimuli (Senior et al., 1991; Unemory et al., 1991; Rasoamanantena et al., 1993). Elastin fragments could be further degraded by non-specific proteinases. It needs to be demonstrated whether such in vivo elastin fragments do contain VGVAPG sequences giving elastin-derived peptides important biological activities.

ACKNOWLEDGEMENTS

This work has been supported by funds provided by University René Descartes Paris V.

REFERENCES

Bertaux, B., Hornebeck, W., Eisen, A. Z. and Dubertret, L. (1991) Growth stimulation of human keratinocytes by tissue inhibitor of metalloproteinases. *J. invest. Dermatol.* **97**, 679–685

Boucaut, J. C., Darnbere, T., Poole, T. J., Aoyama, K. M. and Thiery, J. P. (1984) Biologically active synthetic peptides as probes of embryonic development: a competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cell migration in avian embryos. *J.Cell. Biol.* **99**, 1822–1830

Davidson, J. M., Giro, M., Sutcliffe, M., Zoia, A., Quaglino, D., Liu, J. M., Perkett, E., Meryck, B., Broadley, K. N., Russell, S. and Sephel, G. C. (1990). Regulation of elastin synthesis and biological aspects. Tamburro, A. M. and Davidson, J. M. eds. Congedo editore, pp. 395–405

Ghuysen-Itard, A. F., Robert, L., Gourlet, V., Berr, C. and Jacob, M. P. (1993). Loss of homeostatic mechanisms in polymorphonuclear leukocytes of demented and nondemented elderly sujects. *Gerontology* **39**, 163–169

Ghuysen-Itard, A. F., Robert, L. and Jacob, M. P. (1992). Effet des peptides d'élastine sur la prolifération cellulaire. *C.R. Acad. Sci. Paris*, **t.315, série III**, 473–478

Groult, N., Hornebeck, W., Ferrari, P., Tixier, J. M., Robert, L. and Jacob, M. P. (1991) Mechanism of interaction between skin fibroblasts and elastin: differences between elastin fibres and derived peptides. *Cell Biochem. Funct.* **9**, 171–182

Hinek, A., Rabinovitch, M., Keeley, F., Okamura-Oho, Y. and Callahan, J. (1993) The 67 kD elastin / laminin-binding protein is related to an enzymatically inactive, alternatively spliced form of b-galactosidase. *J. clin. Invest.* **91**, 1198–1205

Homsy, R., Pelletier-Lebon, P., Tixier, J. M., Godeau, G., Robert, L. and Hornebeck, W. (1988) Characterization of human skin fibroblasts elastase activity. *J. invest. Dermatol.* **91**, 472–477

Hornebeck, W., Tixier, J. M. and Robert, L. (1986). Inducible adhesion of mesenchymal cells to elastic fibers: elastonectin. *Proc. Natl. Acad. Sci. USA* **83**, 5517–5520

Jacob, M. P. and Hornebeck, W. (1985) Frontiers of matrix biology (L. Robert, M. Moczar and E. Moczar eds), Karger, Basel, Switzerland, pp. 92–123.

Liotta, L. A., Rao, C. N. and Wener (1986) Biochemical interactions of tumor cells with the basement membrane. *Annu. Rev. Biochem.* **55**, 1037–1057

Mecham, R. P., Hinek, A., Entwisth, R., Wrenn, D. S., Griffin, G. L. and Senior, R. P. (1989) Elastin binds to a multifunctional 67 kilodalton peripheral membrane protein. *Biochemistry* **28**, 3716–3722

Myers, B. A., Dubick, M. A., Reynolds, R. D. and Rucker, R. B. (1985). Effect of vitamin B6 (pyridoxin) deficiency on lung elastin cross-linking in perinatal and weanling rat pups. *Biochem. J.* **229**, 153–160

Perdomo, J. J., Gounon, P., Schaeverbeke, M., Schaeverbeke, J., Groult, V., Jacob, M. P. and Robert, L. (1994). Interactions between cells and elastin fibers: an ultrastructural and immunocytochemical study. *J. Cell Physiol.* **158**, 451–458

Rasoamanantena, P., Moczar, E., Robert, L., Wei, S. M., Godeau, G. and Hornebeck, W. (1993). Protective effect of oleoyl peptide conjugates against elastolysis by neutrophil elastase and kappa-elastin-induced monocyte chemotaxis. *Amer. J. respir. Cell. M. Biol.* **8**, 50–55.

Ruoslahti, E. and Pierschbacher, M. D. (1987) New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491–497

Sandberg, L., Pollman, M., Price, L., Roos, P., Blankenship, J. and Boucek, R. (1990) Structural guidelines for an acceptable elastin and tropoelastin. Tamburro A.M. and Davidson, J.M. eds. *Congedo editore*, pp. 23–44

Senior, R. M., Griffin, G. L., Mecham, R. P., Wrenn, D. S., Prasad, K. U. and Urry, D. W. (1984) Val-Gly-Val-Ala-Pro-Gly, a repeating peptide in elastin, is chemotactic for fibroblasts and monocytes. *J. Cell Biol.* **99**, 870–874.

Senior, R. M., Griffin, G. L., Fliszar, C. L., Shapiro, S. D., Goldberg, G. I. and Welgus, H. G. (1991) Human 92 and 72 kilodalton type IV collagenase are elastases. *J. Biol. Chem.* **266**, 7870–7875.

Uitto, J., Olsen, D. R. and Fazio, M. J. (1989). Extracellular matrix of the skin: 50 years of progress. *J. Invest. Dermatol.* **92**, 61S–77S

Unemory, E. N., Hibbs, M. S. and Amento, E. P. (1991) Constitutive expression of a 92 Kda gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. *J. Clin. Invest.* **88**, 1656–1662.

Varga, Z., Jacob, M. P., Robert, L. and Fülöp, T. (1989). Identification and signal transduction mechanism of elastin peptide receptor in human leukocytes. *FEBS Lett.* **258**, 5–8

Werb, Z., Tremble, P. M., Behrentsen, O., Crowley, E. and Damsky, C. H. (1989) Signal transduction through the fibronectin receptor induces collagenase and stromelysine gene expression. *J. Cell Biol.* **109**, 877–889

Yeh, H., Ornstein-Goldstein, H., Indik, Z., Sheppard, P., Anderson, N., Rosenbloom, J. C., Cicila, G., Yoon, K. and Rosenbloom, J. (1987). Sequence variation of bovine elastin mRNA due to alternative splicing. *Collagen and Rel. Res.* **7**, 235–247