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Identification and signal transduction mechanism of elastin peptide receptor in human leukocytes

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The existence of a novel receptor on human polymorphonuclear leukocytes (PMNLs) and monocytes was demonstrated, named soluble elastin peptide receptor. Soluble elastin peptides, like K-elastin, which are liberated from elastin fibres, can be found in the sera, and they possess several biological activities such as chemotaxis. Studying the effects of elastin peptides on leukocytes, it was found that: (i) the elastin peptide stimulates the oxidative burst, the intracellular free Ca^{2+} elevation through a specific receptor; and (ii) in the signal transduction mechanism of this elastin peptide receptor, the phosphatidylinositol breakdown is involved.

Elastin peptide; Signal transduction mechanism; Phosphatidylinositol breakdown

1. INTRODUCTION

Interaction between cells and macromolecules of the extracellular matrix is critically important on one hand for the maintenance of physiological cellular structure and functions, and the other hand in pathogenesis of several diseases such as emphysema [3,4] and atherosclerosis [5]. During these last few years it became evident that specific interactions exist between different cell types (e.g. smooth muscle cells, fibroblasts and leukocytes) and the degradation products of this matrix [1,2].

During the injury of elastic fibres, soluble elastin peptides are liberated, and these liberated elastin peptides are present in the circulation [4,6]. It was demonstrated that soluble elastin peptides (such as K-elastin, KE) possess important biological activities. They are chemotactic for fibroblasts [7] and for monocytes [8], induce intralysosomal enzyme release from monocytes [9], and stimulate the ^{45}Ca uptake into monocytes, fibroblasts and smooth muscle cells [10]. According to the above-mentioned effects of soluble elastin peptides the aim of our present work was: (i) to study the effects of soluble elastin peptide, KE, on human granulocytes; (ii) to demonstrate the existence of soluble elastin specific receptors on leukocytes; and (iii) to determine the signal transduction mechanism of these elastin peptide receptors.

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2. MATERIALS AND METHODS

2.1. *Patients*

Cells were obtained from 15 healthy young (<35 years) subjects. All of them gave fully informed consent and were volunteers. The selection of these persons was based on the criteria of good physical and mental health confirmed by careful clinical and laboratory investigations.

2.2. *Cell preparation*

Polymorphonuclear leukocytes (PMNLs) and monocytes were separated by Ficoll-Hypaque density gradient centrifugation according to the method of Böyum [11]. The cells were at least 95% viable judged by Trypan blue exclusion.

2.3. *Materials*

Superoxide dismutase (SOD), Cytochrome C, FMLP were from Sigma, Quin 2/AM was purchased from Calbiochem, Pertussis toxin (PT) was from List Biological Laboratories, and myo(2-[^{3}H])inositol was from Amersham (spec. act.: 614 GBq/mmol).

2.4. *K-elastin*

This was prepared from bovine ligamentum nuchae elastin by alkaline hydrolysis in ethanol [12]. The prepared KE was tritiated with NaB^3H_4 according to the method of Bieger and Scheele [13], spec. act.: $6.66.10^6$ cpm/μg.

2.5. *Experimental conditions*

The cells were resuspended in M 199 medium (Gibco) and for measurements in HBSS, with or without 1 mM calcium. The incubations were carried out in a CO_2 incubator, at 37°C. The preincubation time with 500 ng/ml PT was 2 h.

2.6. *O_2^- production*

This was measured by SOD sensitive reduction of Cytochrome C [14].

2.7. *Intracellular free Ca^{2+}*

The PMNLs were loaded with Quin 2/AM according to the method of Tsien et al. [15]. The measurements were carried out as published elsewhere [16].

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2.8. *Myo(2-[^{3}H])inositol loading*

To 10^7 cells/ml, 20 μCi/ml inositol in M 199 medium containing 2% FCS was added. After the appropriate incubation time (2 h, at 37°C), the cells were washed with warm HBSS. After adding KE in 1 μg/ml concentration to the cells, the reaction was stopped with cold TCA at a final concentration of 6%. The lipids were extracted with chloroform:methanol:HCl (50:100:1 v/v/v), the TCA was removed by ethylether extraction and the neutralised aqueous phase was used for inositol phosphate (IP) determination.

2.9. *Separation of inositol phosphates*

The inositol phosphates were separated on DOWEX 1×8 ionexchanger (formate form) with increasing concentration of ammonium formate [17]. The activity of samples was measured in a liquid scintillation counter.

2.10. *[^{3}H]elastin binding assay*

This was based on the method of Smith and Sestili [18]. The cells were incubated with labelled KE in the presence and absence of the excess of unlabelled KE as described in the figure legends. After incubation (2 h, at 22°C), the cells were harvested, and the activity of samples was measured in a liquid scintillation counter.

3. RESULTS

It is well known that one of the obvious signs of PMNL stimulation is the activation of the respiratory burst, therefore, the effect of KE on O_2^- production was studied. In all of our experiments, the FMLP was used as a positive control. It was found that KE stimulates the O_2^- production in PMNLs in a PT-sensitive manner (fig.1).

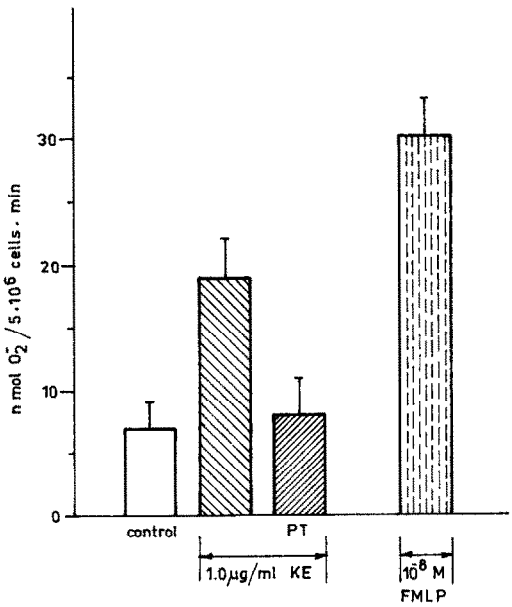

Fig.1. Effect of pertussis toxin, PT (▨), preincubation on K-elastin, KE (▩)-induced O_2^- production in PMNLs. The last column shows the results with the positive control FMLP (▥). Each value represents the mean ± SD of ten determinations.

Previously, it was demonstrated that KE stimulates the calcium influx in monocytes, fibroblasts and smooth muscle cells [10], therefore we were interested to investigate the effect of KE on intracellular free Ca^{2+} levels. It was found that KE stimulates the intracellular free Ca^{2+} elevation (fig.2a), and this KE-stimulated intracellular free Ca^{2+} mobilization can be inhibited by PT preincubation (Fig.2b). The role of both extra-, and intracellular calcium pools, in KE-induced intracellular free Ca^{2+} elevation, was confirmed by measuring the effect of KE on intracellular free Ca^{2+} in the absence of extracellular Ca^{2+}. It can be seen in fig.2c that the KE-induced intracellular free Ca^{2+} elevation decreases 25% in the absence of extracellular Ca^{2+}.

In our experiments, the next step was the determination of [^{3}H]elastin binding specificity. The saturability of [^{3}H]elastin binding to human PMNLs and to monocytes can be seen in fig.3. The results of the Scatchard analysis are shown in the insert of the figure, the calculated $K_d = 0.18$ nM for PMNLs, and $K_d = 0.47$ nM for monocytes.

Because PT inhibits the KE-induced O_2^- production and intracellular free Ca^{2+} mobilisation, the effect of PT preincubation was also studied on [^{3}H]elastin binding (fig.4). It was found that PT preincubation could not alter the binding of [^{3}H]elastin to the receptor.

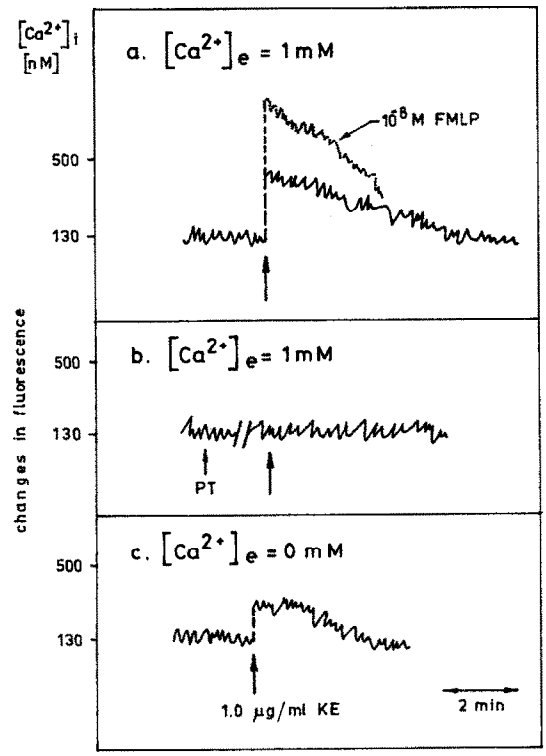

Fig.2. Effect of PT (b) and the absence of extracellular calcium (c) on K-elastin-induced intracellular free calcium mobilization (a). The dotted line in this figure shows the FMLP-induced calcium elevation (a).

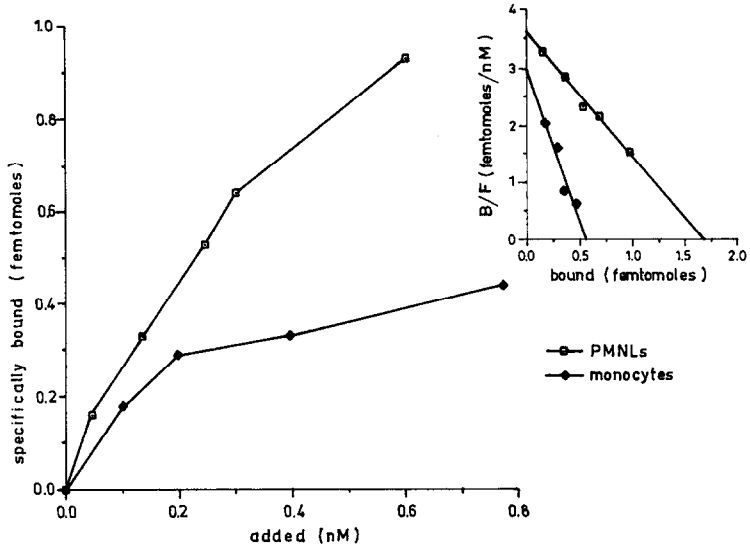

Fig.3. Saturability of [^{3}H]elastin binding to PMNLs (—□—) and monocytes (—■—), at 22°C. Cells were incubated with increasing concentrations of [^{3}H]elastin in the presence and absence of unlabelled elastin (10^{-6} M), the amount of specifically bound KE was calculated from these data. Each experiment was done in triplicate. Scatchard analysis of the binding data is shown in the insert.

From our present results it could be supposed that the elastin peptide receptor belongs to the family of calcium mobilizing receptors. It is well known that the phosphatidylinositol breakdown plays an important role in the signal transduction mechanism of calcium mobilizing receptors, therefore the effect of KE on inositol phosphates formation was studied. In fig.5 it can be seen that KE induces inositol phosphate formation both in PMNLs and in monocytes.

Finally, the effect of PT preincubation on KE-induced inositol phosphate formation was examined. It was found that PT inhibits the KE-stimulated inositol phosphate formation in human leukocytes (fig.6).

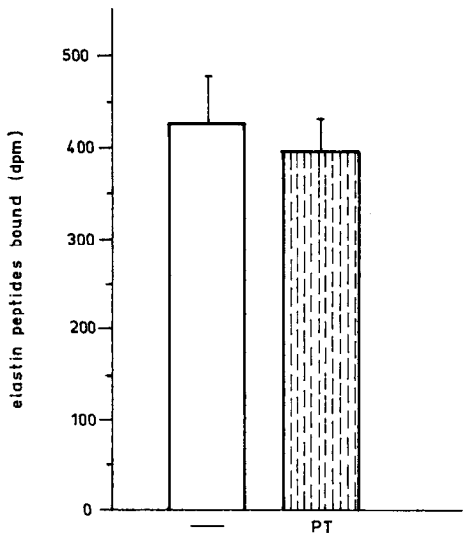

Fig.4. Effect of pertussis toxin, PT (▣), on the binding of [^{3}H]elastin (□), 0.2 nM, to human PMNLs. Each experiment was done in triplicate.

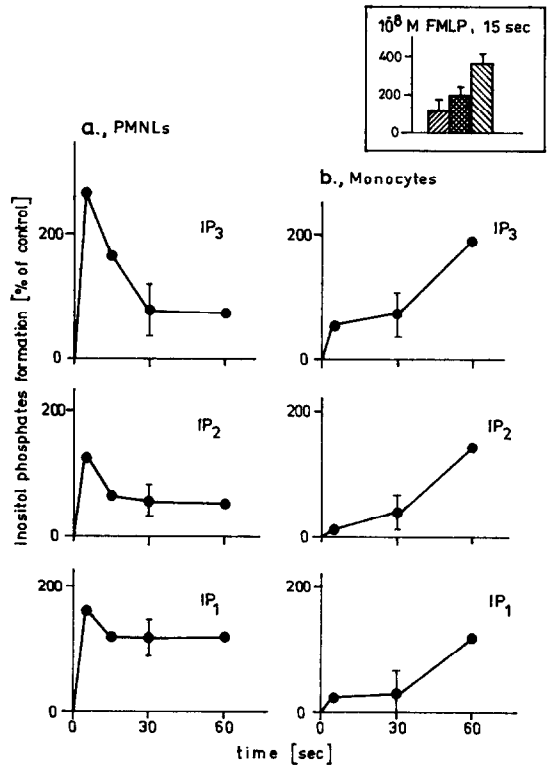

Fig.5. The K-elastin-induced inositol phosphate formation in PMNLs (a) and monocytes (b). Each value represents the mean ± SD of three independent experiments. The insert shows the positive control, FMLP-induced inositol phosphate formation in PMNLs after 15-s incubation.

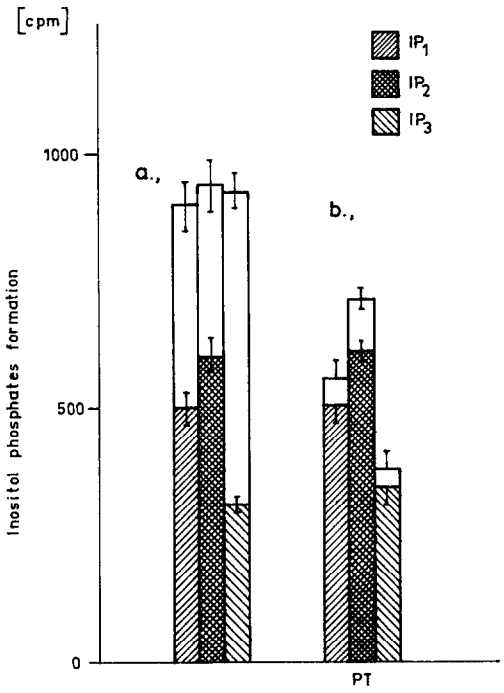

Fig.6. Effect of PT (b) preincubation on K-elastin-induced (a) inositol phosphate formation after 15 s incubation. The ruffled part of columns shows the inositol phosphate level in control cells. Each value represents the mean ± SD of three experiments.

4. DISCUSSION

It is well known that atherosclerosis is a very frequent disease and many efforts have been made to elucidate its pathomechanism. During the atherosclerotic plaque formation, the injury of elastic fibres is occurring and the consequence of this injury is the liberation of soluble elastin peptides. We think that the study of the effects of elastin peptides on leukocytes is important, because leukocytes interact with soluble elastin peptides in physiological circumstances as soluble elastin peptides are present in the sera [4,19].

From our present results it can be concluded that soluble elastin peptides, such as K-elastin, can stimulate human leukocytes, i.e. they induce the O_2^- production, intracellular free calcium mobilization. We were able to demonstrate the presence of elastin peptide specific receptors on human PMNLs and monocytes, with the binding efficiency $K_d = 0.18$ nM for PMNLs and $K_d = 0.47$ nM for monocytes. It was also demonstrated that the phosphatidylinositol breakdown is involved in the signal transduction mechanism of soluble elastin peptide receptor. The receptor is coupled to the phospholipase C through a PT-sensitive GTP binding G protein. This contention is based on the following findings: the PT inhibits the KE-stimulated inositol phosphate formation, O_2^- production, and intracellular free calcium mobilization, but could not alter the binding of [^{3}H]elastin to the cells.

Although the role of soluble elastin peptides liberated from the elastic fibres in the pathogenesis of atherosclerosis is not yet elucidated, we think that the results of our investigation to demonstrate the presence of elastin peptide receptor and its signal transduction mechanism on cells actively participating in the atherosclerotic process [20] will give some new information to atherosclerosis research.

REFERENCES

[1] Yamada, K. (1983) Annu. Rev. Biochem. 52, 761-799.
[2] Watt, F.M. (1986) Trends Biochem. Sci. 11, 482-485.
[3] Crystal, R.G. (1976) The Biochemical Basis of Pulmonary Functions, Marcel Dekker, New York.
[4] Kucich, V., Christner, P., Weinbaum, G. and Rosenbloom, J. (1980) Am. Rev. Resp. Dis. 122, 461-470.
[5] Robert, L., Chaudiere, J. and Jacotot, B. (1984) Regression of Atherosclerosis in Humans, NATO ASI Series, vol. 79. pp. 145-173, Life Sciences.
[6] Kucich, U., Christner, P., Lippmann, M., Fein, A., Goldberg, A., Kimbel, P., Weinbaum, G. and Rosenbloom, J. (1983) Am. Rev. Resp. Dis. 127, S28-S30.
[7] Senior, R.M., Griffin, G.L. and Mecham, R.P. (1980) J. Clin. Invest. 66, 859-862.
[8] Kunitomo, M. and Jay, M. (1985) Inflammation 9, 183-188.
[9] Fülöp, T. jr., Jacob, M.P., Varga, Z., Fóris, G., Leövey, A. and Robert, L. (1986) Biochem. Biophys. Res. Commun. 141, 92-98.
[10] Jacob, M.P., Fülöp, T. jr, Fóris, G. and Robert, L. (1987) Proc. Natl. Acad. Sci. USA 84, 995-999.
[11] Böyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, 77-108.
[12] Kornefeld-Poullain, N. and Robert, L. (1968) Bull. Soc. Chim. 50, 759-771.
[13] Bieger, W. and Scheele, G. (1980) Anal. Biochem. 104, 239-246.
[14] Babior, B.M., Kipnes, R.S. and Curnutte, I.J. (1973) J. Clin. Invest. 52, 741-752.
[15] Tsien, R.Y., Pozzan, T. and Rink, T.J. (1982) J. Cell Biol. 94, 329-334.
[16] Varga, Z., Kovács, É.M., Paragh, G., Jacob, M.P., Robert, L. and Fülöp, T. jr. (1988) Clin. Biochem. 21, 127-130.
[17] Downes, C.P. and Wusteman, M.M. (1983) Biochem. J. 216, 633-640.
[18] Smith, R.G. and Sestili, M.A. (1980) Clin. Chem. 26, 543-550.
[19] Stein, F., Pezess, P.M. and Robert, L. (1965) Nature, Lond. 207, 312-313.
[20] Johanson, L., Holm, J., Skalli, O., Bondjers, G. and Hansson, G.K. (1986) Atherosclerosis 6, 131-139.